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A Practical Synthesis of Kifunensine Analogues as Inhibitors of Endoplasmic Reticulum α-Mannosidase I

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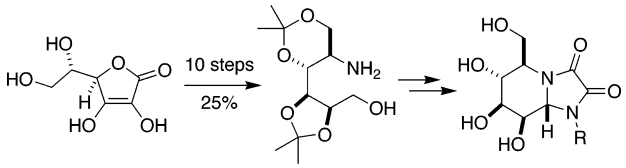

15
L-ascorbic acid

11
key intermediate

kifunensine and
N-1 kifunensine
analogues

A practical synthesis of the potent class I α-mannosidase inhibitor kifunensine (**1**) beginning from the inexpensive and readily available starting material L-ascorbic acid (**15**) is described. The protected amino-alcohol ((2*R*,3*R*,4*R*,5*R*)-5-amino-2,3:4,6-diisopropylidenedioxyhexanol, **11**) served as a key intermediate from which several *N*-1 substituted kifunensine analogues (including *N*-methyl, *N*-cyclohexyl, and *N*-bis(hydroxymethyl)methyl) and 2-desoxakifunensine analogues (including *N*-H and *N*-methyl) were prepared and screened for inhibition of human endoplasmic reticulum α-mannosidase I (ER Man I) and mouse Golgi α-mannosidase IA (Golgi Man IA). In addition, several pseudodisaccharide kifunensine analogues in which a mannose residue was tethered to *N*-1 of kifunensine via a two-, three-, or four-carbon linker and an affinity-bound kifunensine analogue were also prepared and evaluated for biological activity. While the synthesized *N*-1 kifunesine analogues were found to be less potent inhibitors of Class I α-mannosidases than kifuensine itself, the bis(hydroxymethyl)methylkifunensine analogue **6** was shown to selectively inhibit ER Man I over Golgi Man IA.

Introduction

The class I α-mannosidase inhibitor kifunensine (**1**) is a potent, naturally occurring glycosidase inhibitor of Class I (CAZy glycosylhydrolase family 47[1])[2] Kifunensine has been reported to inhibit both human endoplasmic reticulum α-1,2-mannosidase I (ER Man I) and members of the Golgi subfamily of the Class I mannosidases,[3] Golgi α-mannosidase IA, IB, and IC (Golgi Man IA/IB/IC),[4] in addition to Class I mannosidases from plant origin (mung bean α-1,2-mannosidase I (Golgi Man I)) with K_i values of 130 nm, 23 nm, and an IC_{50} value of 20−50 nm, respectively.[4b,5] In addition to aiding in the synthesis and maturation of *N*-linked glycoproteins,[6] ER Man I also plays a key role in the quality control of glycoprotein folding within the endoplasmic reticulum (ER), a process

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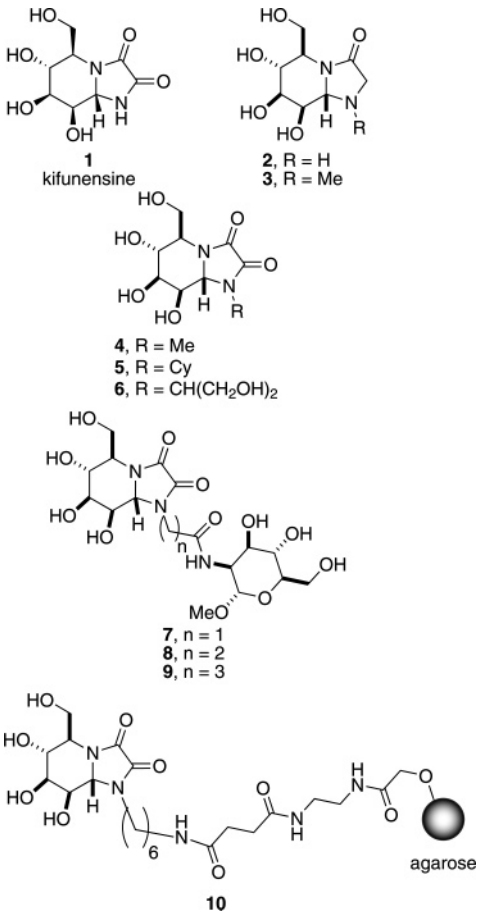

FIGURE 1. Structures of kifunensine and analogues.

that is commonly referred to as endoplasmic reticulum associated degradation (ERAD).[7] A considerable number of genetic diseases are linked to the processes of ERAD.[8] Recently, several studies have demonstrated that inhibition of ER Man I with kifunensine (**1**, Figure 1) or deoxymannojirimycin[9] leads to blockage of the degradation of mutant glycoproteins including the T cell receptor subunit CD3-δ,[10] tyrosinase,[11] α_2-plasmin inhibitor,[12] and α_1-antitrypsin Z variant.[13] In contrast, overexpression of ER Man I was found to accelerate the disposal of misfolded glycoproteins.[14] Surprisingly, acceleration of the "disposal clock" by ER Man I overexpression also triggered a significant disposal of wild-type glycoproteins. Thus a model was proposed in which glycan trimming to $Man_8GlcNAc_2$ moieties in the context of incompletely folded or misfolded polypeptide structures acts as the rate-determining step in generating the signal for glycoprotein disposal.[14a] In this model, ER Man I occupies a unique intersection of both glycoprotein biosynthetic and catabolic pathways and defines the binary decision for the fate of nascent glycoproteins toward folding or disposal. These results suggest that inhibition of ER Man I may serve as a possible approach for the treatment of genetic diseases that occur as a direct result of the processes of ERAD.[5c,14a,15] As a result of its potent inhibition of ER Man I, kifunensine presents an excellent means for the study and regulation of this important enzyme and its role in ERAD. However, kifunensine is also a potent inhibitor of Golgi Man I, an enzyme that is necessary for the maturation of *N*-linked glycoproteins into hybrid and complex type glycoproteins.[4,5a,6] Thus, it would be desirable to generate kifunensine analogues capable of selectively inhibiting ER Man I over Golgi Man I in order to effectively study the mechanism of ER Man I and to aid in the development of novel therapeutic agents for the treatment of genetic diseases related to ERAD. A review of the literature provides several examples in which pseudodisaccharide glycomimetics are used in place of the corresponding monosaccharide moieties to mimic an oligosaccharide in an effort to attain greater potency and/or more selectivity in recognition by a targeted enzyme.[16] Accordingly, we believed that a kifunensine pseudodisaccharide could be developed that would have increased specificity for ER Man I over other class I α-mannosidases. We report herein the synthesis and preliminary biological evaluation of kifunensine, the *N*-1 substituted 2-desoxakifunensine analogues **2** and **3**, and the *N*-1 substituted kifunensine analogues **4**−**11**, which include the tethered disaccharide mimetics **7**−**9** and the affinity bound kifunensine analogue **10** (Figure 1).

Our studies began with an improved synthesis of kifunensine itself. It was our wish to develop a convenient and inexpensive preparation of kifunensine from which intermediates could be used to generate kifunensine analogues that may be useful for the study of class I α-mannosidases. Kifunensine (**1**) was originally isolated by Iwami et al. from the actinomycete *Kitasatosporia kifunensine* no. 9482 and was designated FR900494 in

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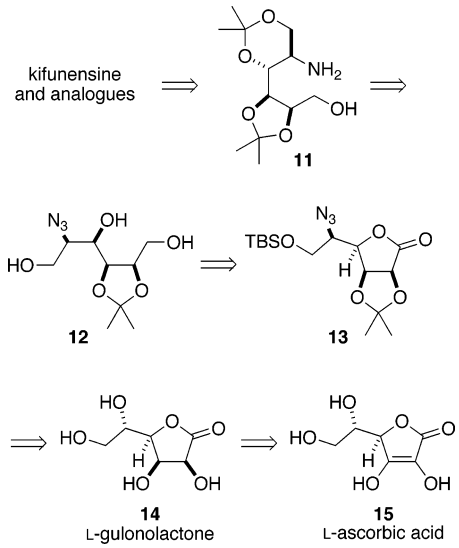
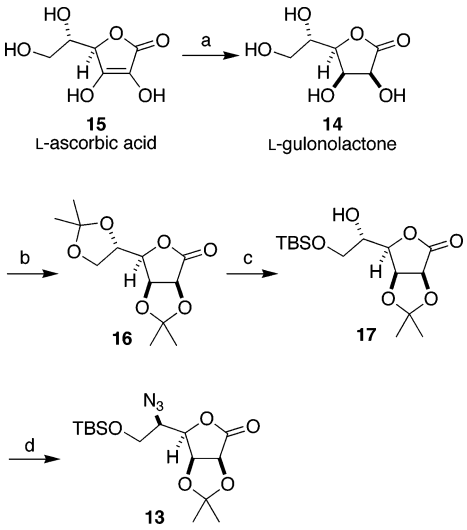
that report.[17] Subsequently, Kayakiri et al. reported the structure and stereochemistry of kifunensine.[18] The structural complexity of the fused heterocyclic ring of kifunensine has limited the synthetic approaches to only two to date.[19] The first synthesis, reported by Kayakiri et al. in the early 1990s, was efficient and high yielding, furnishing kifunensine in eight steps with a 32.5% overall yield beginning from D-mannosamine.[19a,b] More recently, Hudlicky and co-workers reported a formal synthesis of kifunensine beginning from chlorobenzene, which utilizes the same key intermediate and key synthetic step as was first developed by Kayakiri.[19c,d] Of the two approaches, Kayakiri's synthesis presents the most desirable preparation of kifunensine known to date because of its short synthetic sequence and high overall yield. However, the relatively high cost and low availability of D-mannosamine as a starting material remains an unattractive aspect of Kayakiri's synthesis. As a result of our group's long-standing interest in novel preparations of azasugars and azasugar analogues,[20] we chose to undertake a practical synthesis of kifunensine that could also be used to generate novel *N*-1 substituted kifunensine analogues for use in the study of class I α-mannosidases. We report herein a practical synthesis of kifunensine and several *N*-1 substituted kifunensine analogues beginning from the inexpensive and readily available L-ascorbic acid. Our preparation is relatively short and efficient, utilizing simple, highly reproducible steps, and has allowed for the synthesis of multiple gram quantities of **11**, a precursor first utilized in Hulicky's formal synthesis of kifunensine (Scheme 1).[19c,d] This intermediate has been readily transformed into kifunensine and has served as an ideal branching point for the synthesis and preliminary biological evaluation of a variety of novel *N*-1 substituted kifunensine analogues.

A retrosynthetic analysis of our strategy toward kifunensine and analogues is outlined in Scheme 1. In a formal synthesis, Hudlicky has previously obtained a fully protected kifunensine precursor from the amino-alcohol **11**.[19c,d] We envisioned that **11** could also serve as a key intermediate in the synthesis of a variety of *N*-1 substituted kifunensine analogues. Amino-alcohol **11** could be generated in several steps from the triol **12**, which could be readily obtained from reductive ring

SCHEME 1. Retrosynthetic Analysis of Kifunensine and Analogues



SCHEME 2. Synthesis of Azido-lactone 13[a]



[a] Conditions: (a) H_2, 10% Pd/C, H_2O, 50 °C, 7 days (75%); (b) $CuSO_4$, H_2SO_4, acetone (76%); (c) (1) AcOH, H_2O, (2) TBSCl, Im, DMF (87%, 2 steps); (d) HN_3, Ph_3P, DEAD, THF, rt (74%).

opening and deprotection of the known TBS-protected azido-lactone **13**.[21] Azide **13** has been previously synthesized from L-gulonolactone (**14**) by Fleet,[21] and **14** can be readily obtained by palladium-catalyzed hydrogenation of L-ascorbic acid (**15**) using the method of Andrews.[22]

Results and Discussion

1. Practical Synthesis of Kifunensine. The synthesis of kifunensine (**1**) began with preparation of **13** using previously reported methods (Scheme 2). Hydrogenation of L-ascorbic acid (**15**) to L-gulonolactone (**14**) was accomplished in 75% yield by the method of Andrews.[22, 23]

For the next step, L-gulonolactone (**14**) was treated with copper sulfate, sulfuric acid, and acetone using the

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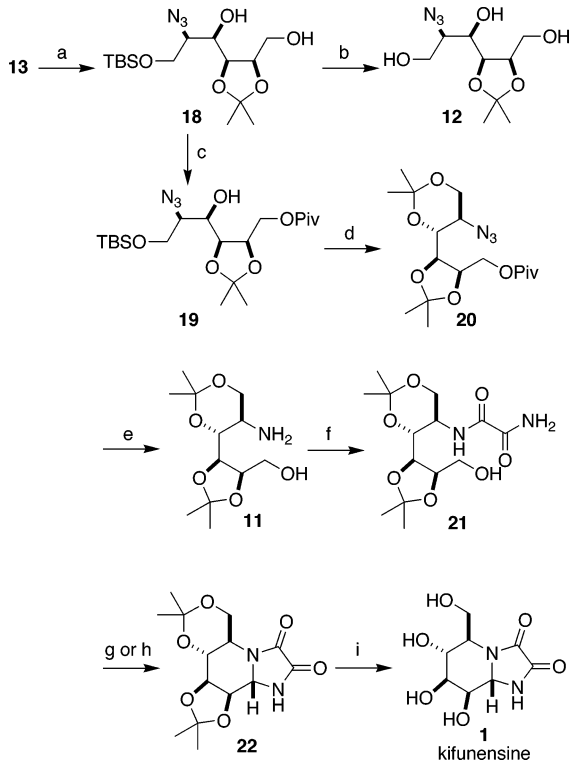
conditions reported by Sano[24] to furnish the desired bis-acetonide **16** in 76% yield.[25] Deprotection of the side chain acetonide of **16** followed by selective protection of the resulting primary alcohol provided silyl-ether **17** in excellent yield.[21] A variety of methods commonly used in the formation of azides from alcohols with inversion of stereochemistry were explored for the conversion of **17** to **13**.[26] The best conditions were determined to be treatment of **17** with hydrazoic acid, triphenylphosphine, and DEAD at room temperature, which provided the azido-lactone **13** in 74% yield. This reaction has been performed on a 15 g scale with no decrease in yield.

Once a high yielding route to azide **13** had been developed, we next explored approaches to Hudlicky's intermediate **11** (Scheme 3).[19c,d] Sodium borohydride reduction of lactone **13** using the conditions reported by Fleet for the selective reduction of a similar azido-lactone[27] furnished diol **18** with the azide intact in excellent yield. Unfortunately, attempts to obtain **11** directly via the triol **12** were unsuccessful due to the instability of **12**.[28] To circumvent this problem, the primary alcohol of **18** was selectively protected as the pivalate ester **19** in 88% yield by treatment with pivaloyl chloride and pyridine. Treatment with TBAF deprotected the silyl ether, and the resulting diol was readily protected to give the acetonide **20**.[29] Lithium aluminum hydride effected reduction of both the azide and ester moieties of **20**, furnishing key intermediate **11** in 98% yield. This amino-alcohol could be crystallized by trituration in ether and proved to be stable at room temperature for at least 1 year.

Finally, to complete the synthesis of kifunensine, EDC coupling of amino-alcohol **11** with oxamic acid in the presence of HOBT afforded Kayakiri's key intermediate **21**.[19b] Intermediate **21** was then subjected to Kayakiri's oxidation/double cyclization protocol[19b] followed by acetonide deprotection to furnish kifunensine (**1**) in 12.2% yield over 14 steps from ascorbic acid. However, we found that the Collins oxidation conditions originally used by Kayakiri et al. for the oxidation/double cyclization of **21** to **22** were not conducive toward analogue synthesis (vida infra). Therefore, alternative oxidation conditions were explored for this substrate. Kayakiri et al. report that the aldehyde resulting from oxidation of **21** is unstable to both silica gel and aqueous workup, and thus only oxidations with a nonaqueous workup could be utilized.[19b] After screening a variety of oxidants with **21** it was found that the oxidation/double cyclization of **21** was best achieved by treatment first with Dess–Martin periodinane and then with excess ammonia in methanol to give protected kifunensine **22** in 89% yield over two steps (Scheme 3).[30] Thus, a total synthesis of kifunensine was completed by our optimized route in 14.2% yield over 14 steps from ascorbic acid.

While our preparation is slightly longer than the previously reported approaches to kifunensine,[19] this synthesis requires only nine purification steps, of which

SCHEME 3. Completion of Kifunensine Synthesis[a]



[a] Conditions: (a) $NaBH_4$, EtOH (87%); (b) TBAF, THF, 0 °C (89%); (c) PivCl, pyr, CH_2Cl_2 (88%); (d) (1) TBAF, THF, −20 °C, (2) 2-methoxypropene, PTSA, CH_2Cl_2 (91%, 2 steps); (e) $LiAlH_4$, Et_2O (98%); (f) (1) oxamic acid, EDC, HOBT, DMF, rt, (2) mp-carbonate resin (93%); (g) (1) CrO_3·pyr, (2) NH_3/MeOH (76%, 2 steps); (h) (1) Dess–Martin periodinane, CH_2Cl_2, (2) NH_3/MeOH (89%, 2 steps); (i) 75% TFA/H_2O (80%).

(23) (a) We found that in our hands the reduction of L-ascorbic acid (**15**) to L-gulonolactone (**14**) could be accomplished in no greater than 75% yield with the reaction taking an average of 7 days at 50 °C under 75 psi hydrogen. The original report by Andrews indicates that this transformation proceeded in 99% yield over 24 h at 50 °C under 50 psi hydrogen (see ref 22). However, in more recent reports, other groups have also noted difficulty reproducing this result (see ref 23b,c). (b) Vekemans, J. A. J. M.; Boerekamp, J.; Godefroi, E. F. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 266–272. (c) Czarnocki, Z.; Mieczkowski, J. B.; Ziolkowski, M. *Tetrahedron: Asymmetry* **1996**, *7*, 2711–2720.

(24) Sano, H.; Sugai, S. *Tetrahedron: Asymmetry* **1995**, *6*, 1143–1150.

(25) We found that treatment of **14** using 2,2-DMP and catalytic PTSA as reported by Fleet (see ref 21) afforded a 1:1 mixture of the desired product **16** and the product resulting from ring opening of the lactone by MeOH generated in the reaction. We were unable to avoid formation of this mixture, thus an alternate method of generating **16** was pursued.

(26) (a) Fleet reported that conversion of the secondary alcohol of **17** to the triflate, followed by displacement with sodium azide furnished the azido-lactone **13** in 76% over two steps (see ref 21). However, we were never able to obtain greater than 30% yield using this two step sequence. Therefore, a variety of conditions were screened for the conversion of **17** to **13**. For the two-step sequence, the use of tetra-*n*-butylammonium azide in place of sodium azide afforded no increase in yield. Thus, a variety of Mitsunobu conditions were explored for the direct conversion of **17** to **13** in one step using diphenylphosphoryl azide (see ref 26b), zinc azide/bispyridine complex (see ref 26c), and hydrazoic acid (see ref 26d). (b) Lal, B.; Pramanik, B. N.; Manhas, M. S.; Bose, A. K. *Tetrahedron Lett.* **1977**, *18*, 1977–1980. (c) Viaud, M. C.; Rollin, P. *Synthesis* **1990**, 130–132. (d) Wolff, H. In *Organic Reactions*; Adams, R., Ed.; Wiley: New York, 1946; Vol. 3, pp 307–336.

(27) Fairbanks, A. J.; Fleet, G. W. J.; Jones, A. H.; Bruce, I.; Al Daher, S.; di Bello, I. C.; Winchester, B. *Tetrahedron* **1991**, *47*, 131–138.

(28) Treatment of TBS-ether **18** with TBAF afforded a highly unstable triol (**12**) in which the acetonide was easily scrambled at room temperature.

(29) Wolfrom, M. L.; Diwadkar, A. B.; Gelas, J.; Horton, D. *Carbohydr. Res.* **1974**, *35*, 87–96.

(30) We observed no formation of the protected kifunensine 8a-epimer of **22** upon oxidation/double cyclization of **21**, as previously reported by Kayakiri et al. (see ref 19b). However, this was likely due to the small scale at which the reactions were performed.

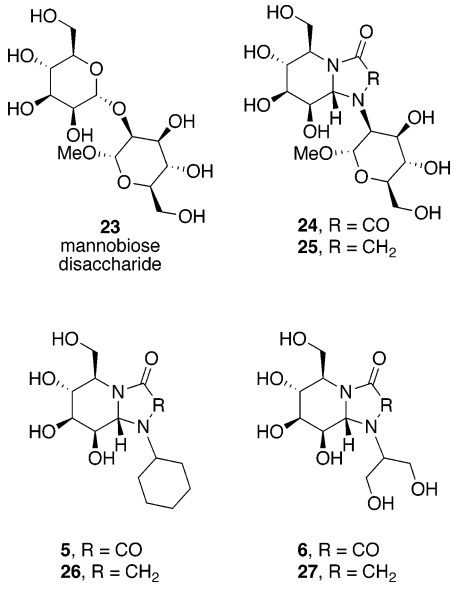


FIGURE 2. Proposed kifunensine analogues.

only five were chromatographic separations. In addition, this synthesis allowed for the generation of gram quantities of the key intermediate **11**. This intermediate was readily transformed into kifunensine using reported methods and provided an excellent branching point for the synthesis of the novel kifunensine analogues presented in the next section.

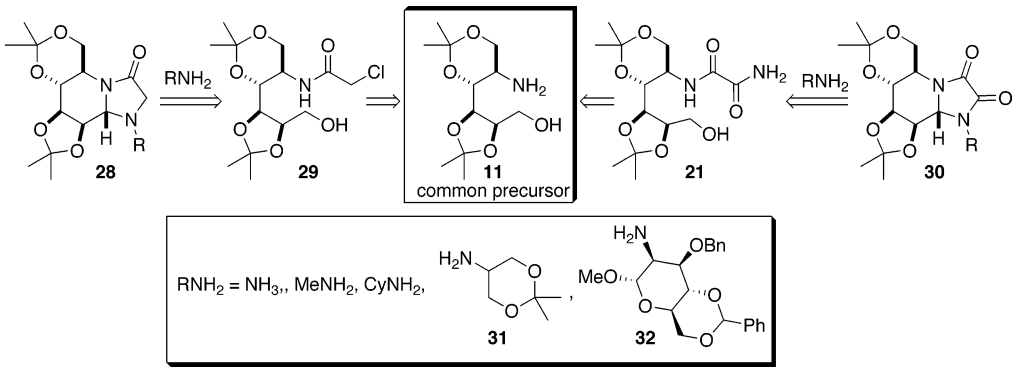
2. Synthesis of Novel *N*-1 Substituted Kifunensine Analogues. In addition to developing a new route to kifunensine, we were also interested in the preparation of novel *N*-1 substituted kifunensine analogues that could be used as biochemical tools for the study of class I α-mannosidases, as well as serve as possible leads in the development of inhibitors that are specific for ER Man I over other class I α-mannosidases. Kifunensine pseudodisaccharide **24** was chosen as our initial synthetic target to mimic mannobiose disaccharide **23**, an ER Man I substrate (Figure 2). We believed that pseudodisaccharide **24** should retain the inhibitory properties of kifunensine in the glycone binding site, allow for additional inhibitor-enzyme interactions within the aglycone binding site, and remain resistant to hydrolysis. It was thought that a compound such as **24** could capitalize upon added interactions within the aglycone binding site allowing it to serve as a better inhibitor of class I α-mannosidases than kifunensine. In addition, **24** could also be useful for mechanistic studies with ER Man I to determine the amino acid residues that serve as the catalytic acid and base within the enzyme active site.[31]

We also planned to synthesize the 2-desoxakifunenesine analogue **25** as another potential ER Man I inhibitor. Compounds such as **25** lacking the *C*-2 carbonyl should be more flexible than the corresponding kifunensine analogues, allowing the desoxa compounds to better mimic the *O*-glycosidic bond in the natural substrate **23** yet remain resistant to hydrolysis.

Retrosynthetically, the kifunensine analogues of the general structure **30** could be generated from an oxidation/double cyclization reaction[32] between Kayakiri's intermediate **21** and a suitable amine (Scheme 4). We envisioned that 2-desoxakifunensine analogues of the general structure **28** could be accessed by oxidation/double cyclization of **29** with the desired amine. Amide **29** could in turn be easily generated by acylation of **11**, a key intermediate in our synthesis of kifunensine. Initially, we planned to prepare cyclohexylkifunensine analogues **5** and **26** and bis(hydroxymethyl)methylkifunensine analogues **6** and **27** in order to test the feasibility of this method for the generation of the more complicated pseudodisaccharides **24** and **25** (Figure 2).

At the outset of the synthesis, little was known about the position of binding of kifunensine within the ER Man I active site. Thus the *N*-1 substituted kifunensine analogues shown in Figure 2 were considered to be excellent choices to serve as suitable mimics for the mannobiose disaccharide **23**. However, during the course of this project, Moremen and colleagues published crystal structures of human ER Man I with kifunensine and deoxymannojirimycin bound within the enzyme active site.[5b] These reports revealed that the *N*-H bond at *N*-1 of kifunensine was pointed toward the active site wall and that the two carbonyls at *C*-2 and *C*-3 were solvent exposed. This information implied that the mannobiose disaccharide is more likely bound within the ER Man I active site in a conformation such as that shown in **23a** (Figure 3), a conclusion that was further bolstered in a recent computational study[33] and confirmed in a recent crystal structure.[15b] Given that this conformation is unattainable by the analogues shown in Scheme 4, we proposed to synthesize kifunensine analogues in which the mannose moiety is tethered to the kifunensine moiety by a linker arm. A review of the literature provides several examples of azasugars tethered to sugar moieties via a variety of linkers to serve as oligosaccharide mimics.[34] Thus, the tethered pseudodisaccharide kifun-

SCHEME 4. Retrosynthesis of Proposed Kifunensine Analogues



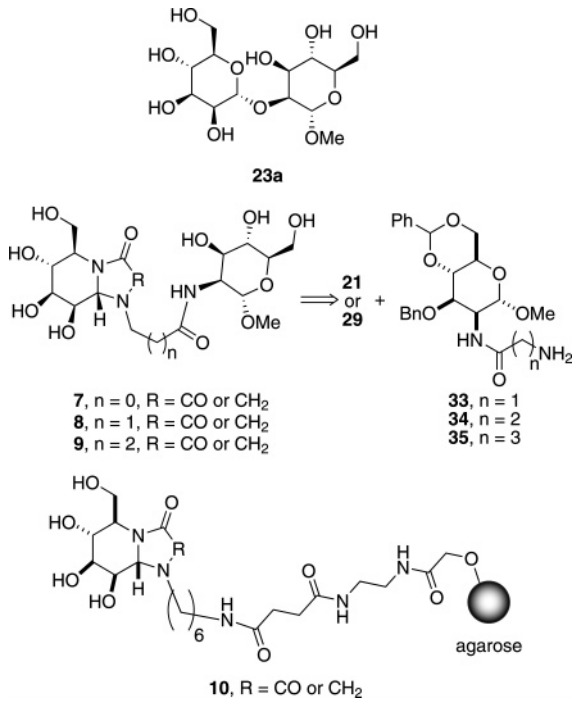
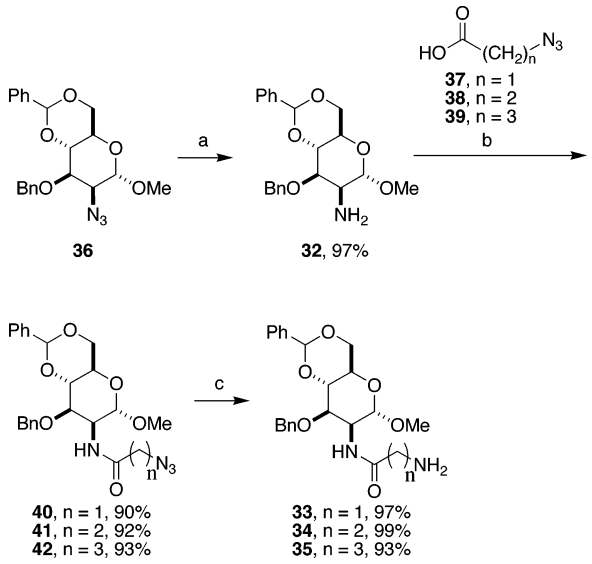


FIGURE 3. Structure of proposed tethered kifunensine analogues.

ensine analogues **7**, **8**, and **9** were chosen as suitable targets.[35] These could be synthesized by the same route described in Scheme 4 using the amino-tethered, protected mannosamines **33**, **34**, and **35** (Figure 3). We envisioned that the variation in tether length would allow for different levels of flexibility in binding for each tethered monosaccharide moiety such that one or more of the analogues **7**−**9** could bind ER Man I in a potent and selective manner.

Finally, the synthesis of an affinity-bound kifunensine analogue such as **10** (Figure 2) was planned for use in the purification of class I α-mannosidases. Many of the glycosidases from the *N*-linked glycoprotein processing pathway are membrane-bound proteins, which are generally more difficult to purify than their soluble counterparts.[36] Mammalian class I α-mannosidases have proven extremely difficult to purify, despite the cloning and expression work accomplished by Moremen.[37] Although deoxymannojirimycin-bound affinity resins have already been successfully used for the purification of pig liver α-mannosidase I,[38] it was thought that a kifunensine-bound affinity resin could provide a more valuable tool for the purification of class I α-mannosidases because kifunensine is approximately 100-fold more potent of an inhibitor of these enzymes than deoxymannojirimycin.[5b] The development of an affinity-bound kifunensine chromatography matrix could simplify the methods required to purify class I α-mannosidases and aid in the discovery of other undetermined class I α-mannosidases located in the golgi and ER. We planned to synthesize **10**, with the tether arm linked to *N*-1 of kifunensine, which corresponds to the position of the glycosyl linkage. This linkage should not interfere with the binding of kifunensine to the enzyme active site as the D-manno configured hydroxyl groups critical for binding would remain unhindered.

SCHEME 5. Synthesis of Amine Starting Materials[a]



[a] Conditions: (a) $LiAlH_4$, Et_2O; (b) EDC, CH_2Cl_2; (c) H_2, 10% Pd/C, EtOAc.

Synthesis of Amine Starting Materials. Before undertaking the synthesis of the kifunensine analogues proposed above, the prerequisite amine starting materials were prepared as shown in Scheme 5. First, treatment of the known azide **36**[39] with lithium aluminum hydride afforded the protected 2-mannosamine **32** in excellent yield. Next, EDC coupling of **32** with 2-azidoacetic acid (**37**),[40] 3-azidopropionic acid (**38**),[41] and 4-azidobutyric acid (**39**)[42] furnished the tethered azides **40**, **41**, and **42**, respectively. Palladium-catalyzed reduction of the azide

(31) (a) Recent crystal structures of ER Man I obtained in the presence of $Man_9GlcNAc_2$ (see 31b), kifunensine, or deoxymannojirimycin (see ref 5b) did not conclusively determine the identity of the catalytic acid and catalytic base required for the hydrolysis of the necessary mannose residue from the oligosaccharide moiety. However, during the course of this project Moremen and colleagues were able to identify the catalytic acid and catalytic base upon determination of a cocrystal structure of ER Man I with a nonhydrolyzable thiodisaccharide pseudosubstrate (see ref 15b). (b) Vallee, F.; F., L.; Yip, P.; Sleno, B.; Herscovics, A.; Howell, P. L. *EMBO J.* **2000**, *19*, 581−588.

(32) For details on the oxidation/double cyclization reaction sequence towards the synthesis of kifunensine, see ref 19b.

(33) Mulakala, C.; Reilly, P. J. *Proteins* **2002**, *49*, 125−134.

(34) For selected examples, see: (a) Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7653−7662. (b) Johns, B. A.; Johnson, C. R. *Tetrahedron Lett.* **1998**, *39*, 749−752.

(35) Examination of molecular models indicated that a tether length of four atoms between kifunensine and the sugar ring would be optimal for mimicking the likely conformation of a disaccharide bound in the ER Man I active site.

(36) (a) Kaushal, G. P.; Elbein, A. D. *Methods Enzymol.* **1989**, *179*, 452−475. (b) Tulsiani, D. R. P.; Touster, O. *Methods Enzymol.* **1989**, *179*, 446−451. (c) Kaushal, G. P.; Elbein, A. D. *Methods Enzymol.* **1994**, *230*, 316−329.

(37) Gonzalez, D. S.; Karaveg, K.; Vandersall-Nairn, A. S.; Lal, A.; Moremen, K. W. *J. Biol. Chem.* **1999**, *274*, 21375−21386.

(38) Schweden, J.; Bause, E. *Biochem. J.* **1989**, *264*, 347−355.

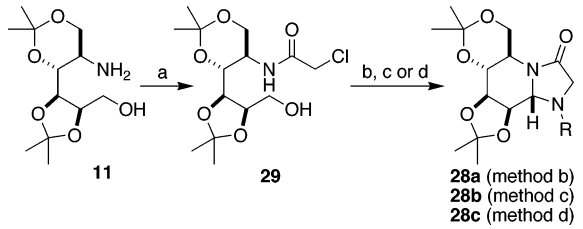
(39) Sugawara, T.; Igarashi, K. *Carbohydr. Res.* **1988**, *172*, 195−207.

(40) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781−783.

(41) Lakanen, J. R.; Coward, J. K.; Pegg, A. E. *J. Med. Chem.* **1992**, *35*, 724−734.

(42) Khoukhi, N.; Vaultier, M.; Carrie, R. *Tetrahedron* **1987**, *43*, 1811−1822.

SCHEME 6. Synthesis of 2-Desoxakifunensine Analogues[a]



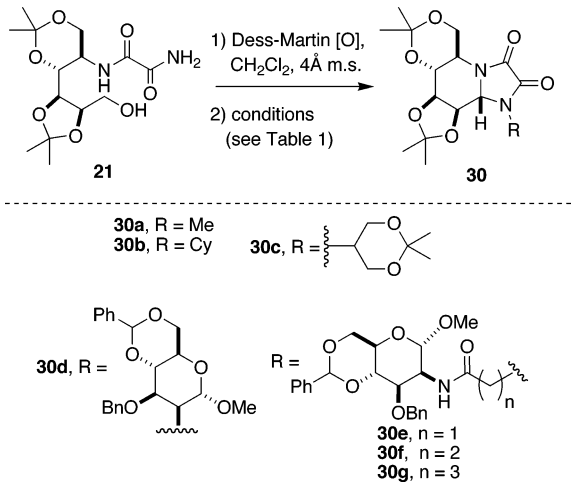
[a] Conditions: (a) $ClCOCH_2Cl$, NaOAc, acetone, H_2O (83%); (b) (1) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, (2) NH_3 (100 equiv), MeOH, rt (79%); (c) (1) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, (2) $MeNH_2$ (100 equiv), MeOH, rt (68%, 2 steps); (d) (1) TPAP, NMO, 4Å m.s., CH_2Cl_2, rt, (2) cyclohexylamine (5 equiv), NaOMe, 4Å m.s., MeOH, reflux (24%, 2 steps).

moieties of **40**, **41**, and **42** then furnished the desired tethered mannosamines **33**, **34**, and **35**, respectively, also in excellent yields.

Synthesis of *N*-1 Substituted, 2-Desoxakifunensine Analogues. The first series of analogues to be investigated were the 2-desoxakifunensine analogues of the general structure **28** shown in Scheme 6. Thus, treatment of **11** with chloroacetyl chloride in the presence of sodium acetate furnished the desired oxidation/double cyclization precursor **29** in good yield. A variety of conditions were attempted for the oxidation of **29** to the corresponding aldehyde, and it was found that the Swern and tetra-*n*-propylammonium perruthenate (TPAP) oxidations with nonaqueous workups provided the best yields of the desired aldehyde. This aldehyde was found to be unstable and was thus used immediately in the double cyclization step without purification. Treatment of the aldehyde with a large excess of ammonia or methylamine in methanol provided good yields of the 2-desoxakifunensine analogues **28a** and **28b**. The cyclohexylamine derivative **28c** proved much more difficult to prepare. Although many conditions were screened,[43] the best results obtained were by treating the crude aldehyde with 5 equiv of cyclohexylamine and sodium methoxide in refluxing methanol. However, a 24% yield of model substrate **28c** was not acceptable for further pursuit of other inhibitors related to this class of analogues.

Synthesis of *N*-1 Substituted Kifunensine Analogues. Next, reaction conditions were explored for the synthesis of kifunensine analogues of the general structure **30** (Scheme 7). Early on, it was discovered that subjecting **21** to a Collin's oxidation followed by double cyclization with 2−5 equiv of amine did not furnish any of the desired products. This was likely due to poisoning of the double cyclization reaction by residual chromium salts from the oxidation step. Given that a synthetically complex, sugar-like amine was ultimately intended for use in the oxidation/double cyclization reaction, alternative oxidation conditions were explored that would allow for the use of 1−2 equiv of amine to accomplish this

SCHEME 7. Synthesis of Kifunensine Analogues



sequence. As mentioned previously, it was found that the Dess−Martin oxidation afforded the best yields of product after the two-step sequence. Using this protocol, protected *N*-methylkifunensine **30a** was prepared in excellent yield (entry 1). For the model system, *N*-cyclohexylkifunensine derivative **30b** was obtained in 54% yield using the conditions shown in entry 2. Thus, the model substrate **30b** was successfully prepared in an acceptable yield using only 2 equiv of amine in the oxidation/double cyclization reaction. The bis(hydroxymethyl)methylkifunensine derivative **30c** was also obtained using the known protected aminodiol **31**[44] with these conditions, albeit in fairly low yield (entry 3).

Given the success obtained with the model system, a synthesis of aza-*N*-disaccharide **30d** was attempted. However, oxidation of **21** followed by treatment of the resulting aldehyde with protected mannosamine **32** using the conditions developed for the model system resulted in no product formation (entry 4). Several conditions were investigated in an attempt to drive the double cyclization to completion without success.[45] We speculate that protected mannosamine **32** is likely too sterically encumbered to undergo the double cyclization reaction with the aldehyde that results from oxidation of **21**.

Next, the synthesis of the tethered kifunensine analogues **30e**, **30f**, and **30g** was attempted. Oxidation of **21** followed by treatment with the appropriate tethered amino sugar **33**, **34**, or **35**, using the conditions developed for the cyclohexylamine model system, resulted in formation of the desired protected kifunensine pseudodisaccharides **30e**, **30f**, and **30g**, respectively (entries 5−7). The reported yields for the synthesis of **30e**−**g** are optimized, indicating that these compounds are sterically and perhaps electronically poor substrates for use in the double cyclization reaction.

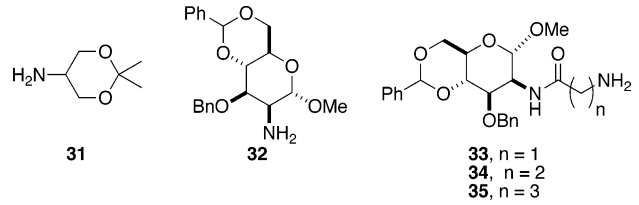
Once the protected kifunensine and 2-desoxakifunensine analogues had been obtained, methods were explored for deprotection of these compounds to the

(43) A variety of other conditions were attempted to generate **28c** including treatment with 100 equiv of cyclohexylamine in a variety of solvents at ambient and elevated temperatures, heating in refluxing toluene, and heating in a variety of solvents under acidic conditions (PTSA, $Sc(OTf)_3$). However, none of these conditions resulted in greater than 10% yields of the desired product.

(44) Harada, H.; Morie, T.; Hirokawa, Y.; Kato, S. *Chem. Pharm. Bull.* **1996**, *44*, 2205−2212.

(45) Conditions attempted included heating the imine in *tert*-butyl alcohol or toluene and treatment of the imine with pyridinium *p*-toluenesulfonate (PPTS), $BF_3{\cdot}OEt_2$ or triethylamine in the presence methylene chloride. However, no product formation was observed with any of these reaction conditions.

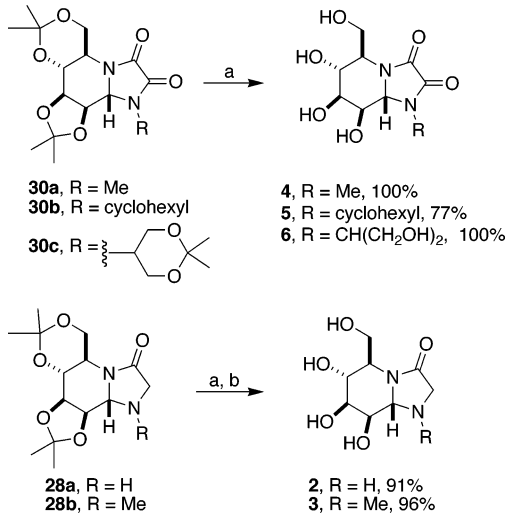
TABLE 1. Conditions Attempted for the Synthesis of 30a–g



entry	reagents (equiv amine)	solvent	temp (°C)	time (h)	product	yield (%)[a]
1	$MeNH_2$ (100)	MeOH	23	16	**30a**	72
2	$CyNH_2$ (2), Et_3N, ui4Å m.s.	CH_2Cl_2	60[b]	16	**30b**	54
3	**31** (2), Et_3N, 4Å m.s.	CH_2Cl_2	60[b]	16	**30c**	22
4	**32** (2), Et_3N, 4Å m.s.	CH_2Cl_2	60[b]	16	**30d**	0
5	**33** (2), Et_3N, 4Å m.s.	MeOH	65	16	**30e**	30
6	**34** (2), Et_3N, 4Å m.s.	MeOH	65	16	**30f**	34
7	**35** (2), Et_3N, 4Å m.s.	CH_2Cl_2	60[b]	16	**30g**	35

[a] Combined yield over two steps. [b] Reaction performed in a sealed tube.

SCHEME 8. Deprotection of Kifunensine and 2-Desoxakifunensine Analogues[a]

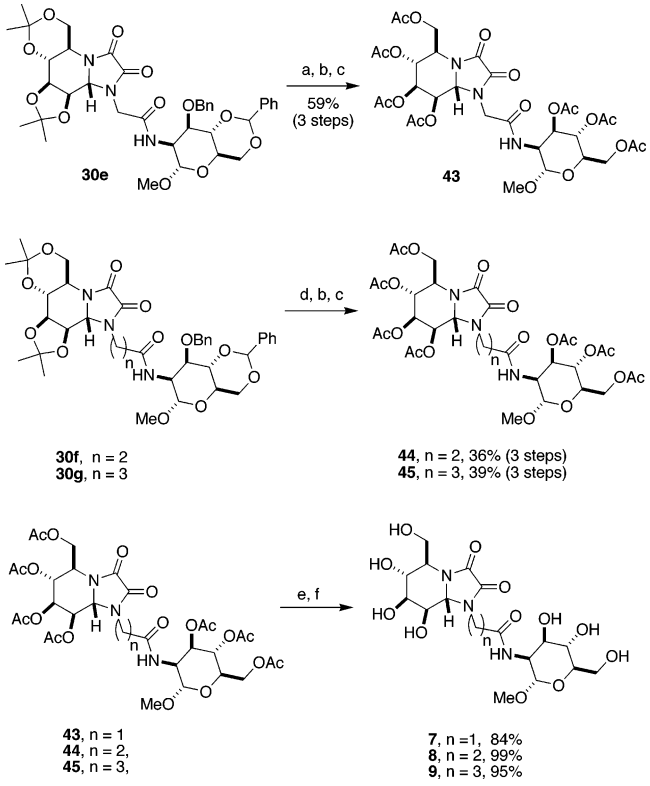


[a] Conditions: (a) 1 N HCl, THF; (b) Dowex 1x8-200 (OH form).

desired azasugar analogues for enzymatic testing. Thus, treatment of kifunensine derivative **30a** with 1 N HCl in THF afforded an excellent yield of *N*-methylkifuensine **4**, previously prepared by Kayakiri (Scheme 8).[19a,b] Next, treatment of **30b** and **30c** with the same conditions resulted in formation of **5** and **6**, respectively, also in excellent yields. The 2-desoxakifunensine derivatives **28a** and **28b** were similarly deprotected and then passed over a short column of Dowex 1x8-200 resin (OH^- form) to obtain the free base analogues **2** and **3**.

The tethered pseudodisaccharide kifunensine analogues **30e–g** proved extremely difficult to deprotect because of the sensitive functionalities present within these compounds (Scheme 9). Different conditions were required for removal of the benzyl groups and benzylidene acetals of **30e** than for **30f** and **30g**. Thus, treatment of **30e** with 10% Pd/C under 45 psi H_2 resulted in complete removal of the benzyl ether and partial removal of the benzylidene acetal. Transfer hydrogenation conditions using Pearlman's catalyst and cyclohexene in refluxing ethanol were necessary to remove the benzyl ether and the benzylidene acetal groups of **30f** and **30g**. In addition, these conditions resulted in partial acetonide removal for both **30f** and **30g**. Because of the partial removal of the benzylidene and isopropylidene acetals of **30e**, **30f**, and **30g** during the debenzylation step, the crude products were taken on directly for acetal removal.

SCHEME 9. Deprotection of Tethered Kifunensine Analogues[a]



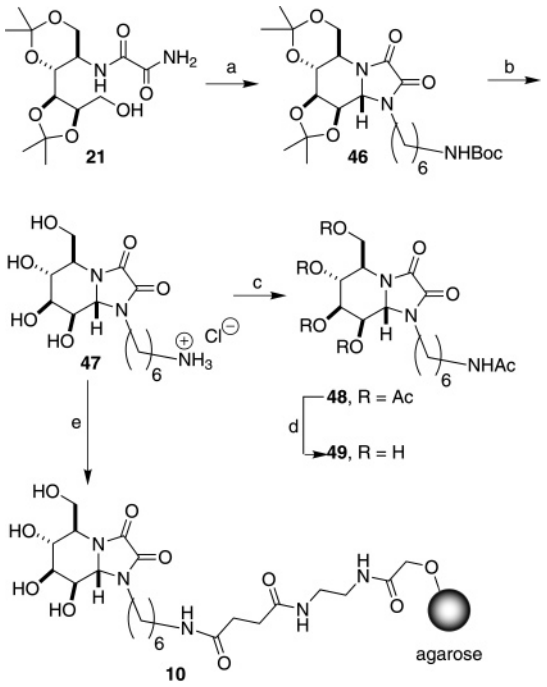
[a] Conditions: (a) 45 psi H_2, 10% Pd/C, MeOH; (b) PTSA, MeOH, ethylene glycol, reflux; (c) Ac_2O, pyr; (d) 20% Pd(OH)2/C, cyclohexene, EtOH, reflux; (e) NaOMe, MeOH; (f) Dowex 50Wx8-100 (pyridinium form).

Several different conditions were attempted for the deprotection of the remaining acetonides, often resulting in a mixture of desired and undesired products.[46] Ultimately, it was found that treatment with PTSA and excess ethylene glycol in refluxing methanol using a modification of the conditions reported by Nicolaou[47] formed the desired crude products. However, these compounds were not amenable to purification by HPLC or chromatography. Therefore, they were peracetylated to furnish moderate yields of **43**, **44**, and **45** over three steps. Removal of the acetates was accomplished by treatment with sodium methoxide in methanol, and the

(46) Treatment with the 1 N HCl/THF conditions used for deprotection of the other kifunensine analogues caused hydrolysis of the methyl glycosidic bond of the attached mannosamine. Alternatively, treatment with refluxing 80% acetic acid in water gave incomplete acetonide removal even after several days. Treatment with 75% trifluoroacetic acid in water, conditions employed by Kayakiri et al. for diacetonide removal in the original kifunensine synthesis (see ref 19b), resulted in hydrolysis of the amide tether, as did treatment with 2% HCl in methanol.

(47) Nicolaou, K. C.; Mitchell, H. J.; Fylaktakidou, K. C.; Rodriguez, R. M.; Suzuki, H. *Chem. Eur. J.* **2000**, *6*, 3116–3148.

SCHEME 10. Synthesis of Affinity-Bound Kifunensine Analogue[a]



[a] Conditions: (a) (1) Dess−Martin [O], CH_2Cl_2, 4Å m.s., (2) $H_2N(CH_2)_6NHBoc$, CH_2Cl_2, Et_3N, 4Å m.s., 60 °C, sealed tube (66%, 2 steps); (b) 1 N HCl, THF (98%); (c) Ac_2O, Et_3N, DMF (69%); (d) NH_3, MeOH (64%); (e) Affigel-10, MeOH, Et_3N, 4 °C (100%).

basic salts were removed by passage over a short column of Dowex 50Wx8-100 resin (pyridinium form) to furnish the desired products **7**, **8**, and **9** in excellent yields.

Synthesis of an Affinity-Bound *N*-1 Linked Kifunensine Analogue. Next the synthesis of an affinity-bound kifunensine analogue was undertaken. Dess−Martin oxidation of oxamido-alcohol **21** followed by treatment of the resulting aldehyde with 2 equiv of commercially available $H_2N(CH_2)_6NHBoc{\cdot}HCl$, using the conditions developed previously in our model system, yielded 66% of the Boc-protected kifunensine analogue **46**. Global deprotection was accomplished by treatment with 1 N HCl in THF to give **47** as the hydrochloride salt. Compound **47** was quantitatively appended to Affi-Gel 10 resin to furnish the desired affinity-bound kifunensine analogue **10**.[48] In the event that the Affi-Gel-bound analogue **10** did not bind to ER Man I, the *N*-acetamido derivative **49** was also prepared to serve as a control for enzyme screening. The *N*-acetamido analogue **49** was prepared by peracetylation of **47** to generate **48**, followed by removal of the acetate protecting groups by treatment with ammonia.

α-Mannosidase I Inhibitory Activities of Kifunensine Analogues. All of the synthetic kifunensine and 2-desoxakifunensine analogues were then screened for inhibitory activity against human ER Man I and mouse Golgi Man IA by determining IC_{50} values for the respective compounds (Table 2).

TABLE 2. Inhibition (IC_{50} Values) of Human ER Man I and Mouse Golgi ManIA by Kifunensine Analogues

	IC_{50} (mM)	
compd	human Er Man I	mouse Golgi Man IA
1	<0.03	<0.03
2	0.03	0.15
3	0.9	1.0
4	0.08	0.17
5	2.6	>5.0
6	0.2	2.1
7	>5.0	>5.0
8	2.1	>5.0
9	2.2	1.8
10[a]	>5.0	nd[b]
49	5.0	nd[b]

[a] Slurries of 0.5%, 5% and 50% were used for the three different concentrations assayed. [b] Not determined.

Although none of our compounds inhibit ER Man I as well as kifunensine (**1**), these data indicates that the *N*-1 substituted kifunensine and 2-desoxakifunensine derivatives do indeed inhibit both human ER Man I and mouse Golgi Man IA. The *N*-H 2-desoxakifunensine analogue **2** and the *N*-methyl kifunensine analogue **4** are the best inhibitors, likely due to the small steric presence around *N*-1 of the kifunensine substructure. Unfortunately, the 2-desoxakifunensine analogues **2** and **3** are relatively poorer inhibitors of ER Man I as compared to kifunensine. This may indicate that the free amine is interacting unfavorably within the glycone binding site or that replacement of the *C*-2 carbonyl with a methylene results in loss of the structural rigidity necessary to hold the fused pyranose ring hydroxyls in the desired $^1C_4$ conformation necessary for activity.[5b] The *N*-cyclohexyl kifunensine and *N*-bis(hydroxymethyl)methyl kifunensine analogues **5** and **6**, respectively, demonstrate significant inhibition of human ER Man I, with the latter compound having a ~10-fold greater selectivity for inhibition of ER man I over Golgi Man IA. This was unexpected given the anticipated steric interactions with active site residues for the *N*-1 substituents of the analogues for both of the Class I mannosidases and the similarities in positions of the active site residues in the +1 and −1 subsites for both enzymes.[5b,49] It appears that the tethered pseudodisaccharide analogues **8** and **9** may indeed be able to bind the attached mannosamine residue in the aglycone binding site as demonstrated by IC_{50} values of ~2 mM. However, **7**, which contains the shortest tether, does not inhibit human ER Man I to any considerable degree. Similarly, both the Affi-Gel linked kifunensine inhibitor **10** and the tethered amide **49** were poor inhibitors of human ER Man I even at the highest concentrations tested. Thus, the affinity bound kifunensine analogue **10** would likely not be useful for the purification of class I α-mannosidases. Given the fact that the tethered pseudodisaccharide kifunensine analogues **8** and **9** exhibited reasonable inhibition of ER Man I, it is interesting that the *N*-hexylamido kifunensine analogue **49** exhibited such poor inhibition (IC_{50} value of ~5 mM). This may indicate that the amide functionality exhibits an unfavorable interaction within the aglycone binding site, in which case a switch to either an ether or ester linkage

(48) Coupling procedures may be found in the booklet *Activated Immunoaffinity Supports* and *Bulletin 1085*, available from Bio-Rad Laboratories, Inc., Life Sciences Group, 2000 Alfred Nobel Drive, Hercules, CA 94547.

(49) Tempel, W.; Karaveg, K.; Lui, Z.-J.; Rose, J.; Wang, B.-C.; Moremen, K. W. *J. Biol. Chem.* **2004**, *279*, 29774−29786.

may improve the inhibitory properties of these kifunensine analogues.

In summary, a practical synthesis of kifunensine has been accomplished using L-ascorbic acid as an inexpensive and readily available starting material. The synthetic route allowed for the synthesis of a number of novel *N*-1 substituted kifunensine and 2-desoxakifunensine derivatives including an affinity-bound kifunensine analogue. Preliminary biological assays of the reported compounds with human ER Man I and mouse Golgi Man IA yielded varying results, with none of the compounds exhibiting inhibition comparable to kifunensine alone. However, the improved specificity for the *N*-bis(hydroxymethyl)methyl kifunensine analogue **6** and the reasonable affinity for the tethered kifunensine pseudodisaccharides **8** and **9** show promise that future *N*-1 substituted kifunensine analogues may be developed that are capable of possessing selectivity in the binding of class I α-mannosidases. Such compounds could enhance our understanding of the role that ER Man I plays in the degradation of misfolded proteins within the ER and may be useful for the treatment of genetic diseases related to ERAD.

Experimental Section

For general experimental details, see Supporting Information.

(3*S*,4*S*,5*R*)-5-[(1′*R*)-1′-Azido-2′-*tert*-butyldimethylsilanyloxyethyl]-3,4-isopropylidenedioxytetrahydrofuran-2-one (13). As an alternate method of preparation, the known title compound[21] was synthesized by Mitsunobu reaction using freshly prepared hydrazoic acid.[26d] **Caution:** Hydrazoic acid should be handled with extreme care. All work involving hydrazoic acid solutions were carried out in an efficient fume hood. The solution was transferred by cannula and any excess hydrazoic acid was quenched by the addition of 10% NaOH. Diethylazodicarboxylate (3.61 mL, 3.99 g, 22.9 mmol) and hydrazoic acid (98.0 mL of a 0.86 M solution in toluene, 84.3 mmol) were added to a solution of triphenylphosphine (22.11 g, 84.28 mmol) in THF (280 mL) at room temperature and stirred for 1 min. Then a solution of the known alcohol **17**[21] (14.01 g, 42.14 mmol) in THF (140 mL) was cannulated into the reaction mixture and stirred for 20 h. The reaction was quenched with the addition of methanol and concentrated to yield a light orange oil. The residue was triterated in 30% EtOAc/hexanes, filtered and concentrated to give a yellow oil. Chromatography (10% EtOAc/hexanes) afforded 9.20 g (61%) of the title compound as a white crystalline solid. We discovered that the literature data for **13** was misreported.[21] Thus, full characterization was obtained for **13**. Data for **13**: mp = 84−85 °C; R_f = 0.66 (30% EtOAc/hexanes); $[\alpha]^{23}_D = -11.0°$ (*c* 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.90 (dd, *J* = 3.3, 5.1 Hz, 1H), 4.85 (d, *J* = 5.1 Hz, 1H), 4.41 (dd, *J* = 3.3, 9.9 Hz, 1H), 4.07 (dd, *J* = 2.4, 10.8 Hz, 1H), 3,87 (dd, *J* = 5.7, 10.8 Hz, 1H), 3.76 (ddd, *J* = 2.4, 5.5, 9.9 Hz, 1H) 1.49 (s, 3H), 1.44 (s, 3H), 0.92 (s, 9H), 0.11 (app d, *J* = 1.4 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 114.3, 75.9, 75.8, 75.4, 63.1, 60.4, 26.8, 25.9, 25.7, 18.2, −5.6, −5.7; IR (neat) 2105 (s), 1796 (s) cm^{-1}; HRMS (ES) calcd for $C_{15}H_{27}N_3NaO_5Si$ (M + Na) 380.1618, found 380.1616. Anal. Calcd for $C_{15}H_{27}N_3O_5Si$: C, 50.40; H, 7.61; N, 11.75. Found: C, 50.31; H, 7.60; N, 11.86.

(2*R*,3*R*,4*R*,5*R*)-5-Azido-6-*tert*-butyldimethylsilanyloxy-2,3-isopropylidene-dioxy-1,4-hexanediol (18). The title compound was prepared by the method of Fairbanks for the reduction of azide-containing γ-lactones to the corresponding diol.[27] Sodium borohydride (1.95 g, 51.5 mmol) was added to a solution of azide-containing γ-lactone **13** (9.20 g, 25.8 mmol) in ethanol (400 mL) at room temperature and stirred for 4 h. The reaction was quenched with the addition of an excess of ammonium chloride with effervescence, filtered and concentrated to yield an oily white solid. Chromatography through a plug of silica (30% EtOAc/hexanes) afforded 8.25 g (89%) of the title compound as a clear oil. Data for **18**: R_f = 0.03 (30% EtOAc/hexanes); $[\alpha]^{23}_D = -43.8°$ (*c* 1.58, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.41 (dd, *J* = 1.4, 7.3 Hz, 1H), 4.30 (dt, *J* = 4.4, 7.3 Hz, 1H), 4.09 (dd, *J* = 3.1, 10.6 Hz, 1H), 3.90−3.85 (m, 2H), 3,80 (dd, *J* = 4.4, 12.4 Hz, 1H), 3.62 (dd, *J* = 1.4, 9.1 Hz, 1H) 3.48 (ddd, *J* = 3.1, 6.3, 9.1 Hz, 1H), 1.53 (s, 3H), 1.41 (s, 3H), 0.92 (s, 9H), 0.11 (app d, *J* = 2.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 108.4, 75.5, 68.3, 64.2, 64.0, 60.9, 26.7, 25.7, 24.7, 18.2, −5.6, −5.7; IR (neat) 3414 (br m), 2100 (s) cm^{-1}; MS (EI) *m/z* (rel int) 346 (M − CH_3, 4), 328 (9), 246 (24), 131 (40), 116 (83), 73 (100), 59 (94); HRMS (DCI w/ NH_3) calcd for $C_{15}H_{32}N_3O_5Si$ (M + H) 362.2111, found 362.2113.

(2*R*,3*R*,4*R*,5*R*)-5-Amino-2,3:4,6-diisopropylidenedioxyhexanol (11). By an alternate method of preparation for the known title compound,[19c,19d] lithium aluminum hydride (1.94 g, 51.2 mmol) was added to a solution of azido-ester **20** (6.34 g, 17.1 mmol) in Et_2O (400 mL) at room temperature and stirred 1 h. The reaction was quenched with successive addition of water (1.95 mL), 10% NaOH (1.95 mL), and water (3.90 mL), the ether was poured off, and the solid was extracted with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated to give an oily yellow solid, which was triterated in hot ether and filtered to yield 3.99 g (89%) of the title compound as a pale yellow solid. The crude material was sufficiently pure for characterization. Data for **11**: mp = 95.5−96.5 °C; R_f = 0.21 (10% MeOH/CH_2Cl_2); $[\alpha]^{23}_D = -38.7°$ (*c* = 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.50 (dd, *J* = 2.3, 6.7 Hz, 1H), 4.29 (dt, *J* = 4.8, 6.7 Hz, 1H), 3.89 (dd, *J* = 5.3, 11.5 Hz, 1H), 3.80, (dd, *J* = 4.8, 12.0 Hz, 1H), 3.76 (dd, *J* = 4.8, 12.0 Hz, 1H), 3.53 (dd, *J* = 2.3, 9.3 Hz, 1H), 3,50 (dd, *J* = 9.3, 11.5 Hz, 1H), 3.11 (td, *J* = 5.3, 9.3 Hz, 1H), 1.85 (br s, 3H), 1.53 (s, 3H), 1.48 (s, 3H), 1.43 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 108.7, 98.9, 77.7, 74.7, 73.9, 66.2, 61.3, 45.9, 28.4, 26.6, 25.7, 19.3; IR (neat) 3355 (br m), 3287 (m), 1606 (w) cm^{-1}; MS (EI) *m/z* (rel int) 246 (M − CH_3, 10), 72 (18), 59 (22), 43 (100); HRMS (ES) calcd for $C_{17}H_{29}N_3NaO_6$ (M + Na) 284.1474, found 284.1463.

(2*R*,3*R*,4*R*,5*R*)-2,3:4,6-Diisopropylidenedioxy-5-oxamoylaminohexanol (21). By an alternate method of preparation for this known compound,[19b] 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (333 mg, 1.74 mmol) was added to a solution of oxamic acid (104 mg, 1.16 mmol), 1-hydroxybenzotriazole hydrate (236 mg, 1.74 mmol), and amino-alcohol **11** (304 mg, 1.16 mmol) in DMF (5.8 mL) and stirred for 1 h at room temperature. Then macroporous triethylammonium methylpolystyrene carbonate resin (mp-carbonate, Argonaut Technologies) (1.83 g, 5.23 mmol, of 2.85 meq/g) was added in one portion and the reaction was stirred for an additional 3 h. The resin was removed by vacuum filtration and washed with CH_2Cl_2, and the filtrate was concentrated to yield a viscous, yellow oil. Chromatography (10:1:0.1 EtOAc/hexanes/Et_3N) afforded 358 mg (93%) of the title compound as an amorphous white solid. ^{1}H NMR and ^{13}C NMR spectral data for **21** matched published reports.[19b]

(3a*S*,3b*S*,6b*R*,10a*R*,10b*S*)-2,2,9,9-Tetramethyl-1,3,8,10-tetraoxacyclohexa-[*e*]cyclopenta[g]-5,6-dione-4-azaindolizidine (22). By an alternate method of preparation for this known compound,[19b] Dess−Martin periodinane (31 mg, 0.072 mmol) was added to a solution of alcohol **21** (16 mg, 0.048 mmol) in CH_2Cl_2 (1.0 mL) at room temperature and stirred for 4 h. The reaction was then diluted with ether (4 mL), filtered through Celite and concentrated to yield the intermediate aldehyde as a clear oil. The crude intermediate aldehyde was dissolved in 7.0 N NH_3−MeOH (0.70 mL, 4.9 mmol NH_3) and stirred at room temperature for 24 h. The reaction was then concentrated to yield a yellow oil. Chromatography (75% EtOAc/hexanes) afforded 13 mg (89%) of the title compound

as a white solid. ^{1}H NMR and ^{13}C NMR spectral data for **22** matched published reports.[19b]

(2*R*,3*R*,4*R*,5*R*)-*N*-(2,3:4,6-Diisopropylidenedioxyhexanol-5-yl)-2′-chloro-acetamide (29). Chloroacetyl chloride (249 μL, 4.0 M solution in acetone, 0.998 mmol) was added dropwise with stirring to a solution of amino-alcohol **11** (261 mg, 0.998 mmol) and sodium acetate (164 mg, 2.00 mmol) in a mixture of 1.4 mL of acetone and 0.7 mL of water at 0 °C. The reaction was allowed to slowly come to room temperature and stirred for 22 h, at which point it was shown to be incomplete by TLC. The reaction was cooled to 0 °C, chloroacetyl chloride (125 μL, 4.0 M solution in acetone, 0.499 mmol) was added dropwise, and the resulting mixture was then allowed to come to room temperature. After stirring for 1 h the reaction was concentrated, and the residue was diluted with water and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to yield 280 mg (83%) of the title compound as an amorphous white solid. The crude material was sufficiently pure for characterization. Data for **29**: R_f = 0.61 (10% MeOH/CH_2Cl_2); $[\alpha]^{23}_D$ = −58.7° (*c* 1.03, CH_2Cl_2); ^{1}H NMR (benzene-d_6, 400 MHz) δ 6.26 (d, *J* = 8.4 Hz, 1H), 4.33−4.24 (m, 1H), 4.22 (d, *J* = 6.6 Hz, 1H), 4.14−4.09 (m, 1H), 3.89 (d, *J* = 9.5 Hz, 1H), 3.81−3.69, (m, 3H), 3.56 (dd, *J* = 8.1, 11.7 Hz, 1H), 3.45 (s, 2H), 1.60 (s, 3H), 1.33 (s, 6H),1.28 (s, 3H); ^{13}C NMR (benzene-d_6, 100 MHz) δ 166.0, 109.5, 99.8, 78.3, 76.1, 70.3, 62.4, 62.0, 47.4, 42.9, 28.1, 27.4, 26.3, 20.6; IR (neat) 3286 (m), 1665 (s) cm^{-1}; MS (EI) *m/z* (rel int) 322 (M − CH_3, 15), 264 (27), 148 (58), 119 (100); HRMS (ES) calcd for $C_{14}ClH_{24}NaNO_6$ (M + Na) 360.1190, found 360.1191.

(3a*S*,3b*S*,6b*R*,10a*R*,10b*S*)-2,2,9,9-Tetramethyl-1,3,8,10-tetraoxacyclohexa-[*e*]cyclopenta[*g*]-6-one-4-azaindolizidine (28a). Dimethyl sulfoxide (11 μL, 12 mg, 0.16 mmol) was added with stirring to a solution of oxalyl chloride (39 μL, 2.0 M solution in CH_2Cl_2, 0.078 mmol) in CH_2Cl_2 (1.0 mL) at −78 °C. After 30 min, a solution of alcohol **29** (24 mg, 0.071 mmol) in CH_2Cl_2 (0.4 mL) was added dropwise and the reaction stirred at −78 °C for an additional 30 min. Triethylamine (50 μL, 36 mg, 0.36 mmol) was then added at −78 °C, and the reaction was stirred for a further 20 min, warmed to room temperature and concentrated to yield an intermediate aldehyde/aminal as an oily, pale yellow solid. The crude material was unstable to chromatography, although the presence of a 1:2 mixture of the aldehyde and aminal was verified by resonances of δ 9.64 (d, *J* = 1.6 Hz, 0.33H) and δ 5.52 (br s, 0.67H), respectively, in the ^{1}H NMR spectrum ($CDCl_3$, 400 MHz). The crude residue was treated with 7.0 N NH_3−MeOH (1.0 mL, 7.0 mmol NH_3) and stirred for 20 h at room temperature. The reaction was concentrated to yield an oily, brown solid. Chromatography (2% MeOH/CH_2Cl_2) afforded 17 mg (79%) of the title compound as a white solid. Data for **28a**: mp = 190−192 °C; R_f = 0.18 (100% EtOAc); $[\alpha]^{23}_D$ = −71.4° (*c* = 0.74, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.64 (d, *J* = 8.6 Hz, 1H), 4.53 (dd, *J* = 5.1, 10.8 Hz, 1H), 4.26 (t, *J* = 8.6 Hz, 1H), 4.06 (dd, *J* = 8.6, 12.3 Hz, 1H), 4.04 (t, *J* = 8.6 Hz, 1H), 3.71 (t, *J* = 10.8 Hz, 1H), 3.54 (ABq, *J* = 16.5, Δν = 87.1 Hz, 2H), 3.46−3.39 (m, 1H), 1.55 (s, 3H), 1.53 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.2, 110.7, 99.8, 77.5, 75.9, 72.9, 71.1, 62.2, 48.1, 47.9, 29.1, 26.5, 23.9, 19.0; IR (neat) 3338 (w), 1695 (s) cm^{-1}; MS (EI) *m/z* (rel int) 298 (M$^+$, 14), 283 (24), 240 (39), 110 (100), 85 (37); HRMS (ES) calcd for $C_{15}H_{26}N_2NaO_6$ (M + Na + MeOH) 353.1689, found 353.1697. Stereochemical assignment for **28a** was determined on the basis of COSY analysis and comparison of the observed *J*-coupling between H_8 (4.04 ppm, t, 8.6 Hz) and H_{8a} (4.64 ppm, d, 8.6 Hz) to those reported in the literature for the closely related structures of kifunensine diacetonide (*J*-coupling between H_8 and H_{8a} of 8.0 Hz) and 8a-epi kifunensine diacetonide (*J*-coupling between H_8 and H_{8a} of 3.0 Hz).[18,19b]

(5*R*,6*R*,7*S*,8*S*,8a*S*)-*N*-(6,7,8-Triacetoxy-5-acetoxymethyl-1-{[1′*S*,2′*S*,3′*R*,4′*S*,5′*R*]-3′,4′-diacetoxy-5′-acetoxymethyl-1′-methoxytetrahydropyran-2′-ylacetamide-2″-yl})-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione (43). A solution of benzyl ether **30e** (49 mg, 0.068 mmol) in MeOH (1.4 mL) was transferred to a Parr bottle and 10% palladium on carbon (10 mg) was added to the solution. The Parr bottle was assembled onto a Parr Shaker, evacuated via an aspirator, and purged first with nitrogen (2×) and then hydrogen (2×). Hydrogenation was carried out under 45 psi of hydrogen at room temperature with vigorous shaking. After 48 h, analysis of the reaction by LCMS indicated complete removal of the benzyl ether so the hydrogen was evacuated, the mixture filtered through Celite and concentrated to obtain a clear oil. The residue was dissolved in dry MeOH (1.4 mL) and ethylene glycol (28 μL, 32 mg, 0.51 mmol) and *p*-toluenesulfonic acid (13 mg, 0.068 mmol) were added. The reaction was stirred at reflux for 2 h, at which point analysis by LCMS indicated that all of the acetonides had been removed. Therefore, the reaction was cooled to room temperature, quenched with pyridine (30 μL) and concentrated to give a pale yellow oil. The oil was dissolved in pyridine (0.7 mL) and acetic anhydride (0.7 mL) and stirred at room temperature for 16 h. The reaction was then concentrated to yield a pale yellow oil. Normal phase HPLC (95% EtOAc/hexanes, 10 mL/min, t_R = 20.4 min) afforded 31 mg (59%) of the title compound as a clear oil. Data for **43**: R_f = 0.39 (100% EtOAc); $[\alpha]^{23}_D$ = 13.1° (*c* 0.52, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.70 (d, *J* = 9.1 Hz, 1H), 5.53 (t, *J* = 3.1 Hz, 1H), 5.42 (d, *J* = 9.5 Hz, 1H), 5.31 (dd, *J* = 4.4, 9.5 Hz, 1H), 5.11−5.01 (m, 3H), 4.77 (dd, *J* = 5.5, 9.6 Hz, 1H), 4.68 (d, *J* = 1.4 Hz, 1H), 4.60−4.53 (m, 2H), 4.33 (ABq, *J* = 16.5, Δν = 65.3 Hz, 2H), 4.33−4.22 (m, 2H), 4.06 (dd, *J* = 2.5, 12.1 Hz, 1H), 3.98 (ddd, *J* = 2.5, 5.9, 9.2 Hz, 1H), 3.41 (s, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 169.8, 169.8, 169.0, 168.8, 168.5, 166.6, 158.3, 156.6, 99.6, 72.0, 69.2, 68.0, 67.2, 67.0, 66.3, 64.1, 62.7, 60.4, 55.3, 52.4, 50.6, 45.8, 20.7 (2 carbons), 20.6 (4 carbons), 20.6; IR (neat) 3334 (br w), 1751 (vs), 1697 (m), 1223 (vs), 1050 (s) cm^{-1}; MS (EI) *m/z* (rel int) 728 (M − OMe, <1), 640 (4), 566 (19), 524 (67), 242 (17), 43 (100); HRMS (ES) calcd for $C_{31}H_{41}N_3NaO_{19}$ (M + Na) 782.2232, found 782.2195.

(5*R*,6*R*,7*S*,8*S*,8a*S*)-*N*-(6,7,8-Triacetoxy-5-acetoxymethyl-1-{[1′*S*,2′*S*,3′*R*,4′*S*,5′*R*]-3′,4′-diacetoxy-5′-acetoxymethyl-1′-methoxytetrahydropyran-2′-ylpropion-amide-3″-yl})-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione (44). 20% palladium hydroxide on carbon (13 mg) was added to a solution of benzyl ether **30f** (50 mg, 0.068 mmol) and cyclohexene (0.35 mL) in EtOH (1.4 mL) and refluxed with stirring for 4 h. At this point, analysis of the reaction by LCMS indicated complete removal of the benzyl ether so it was cooled to room temperature, filtered through Celite and concentrated to obtain a white oil. The residue was dissolved in dry MeOH (1.4 mL), and ethylene glycol (19 μL, 21 mg, 0.34 mmol) and *p*-toluenesulfonic acid (13 mg, 0.068 mmol) were added. The reaction was stirred at reflux for 2 h at which point analysis by LCMS indicated that all of the acetonides had been removed. Therefore, the reaction was cooled to room temperature, quenched with pyridine (20 μL), and concentrated to give a clear oil. The oil was dissolved in pyridine (0.7 mL) and acetic anhydride (0.7 mL) and stirred at room temperature for 16 h. The reaction was then concentrated to yield a clear oil. Normal phase HPLC (95% EtOAc/hexanes, 10 mL/min, t_R = 29.2 min) afforded 19 mg (36%) of the title compound as a clear oil. Data for **44**: R_f = 0.35 (100% EtOAc); $[\alpha]^{23}_D$ = 19.7° (*c* 0.94, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.34 (d, *J* = 8.8 Hz, 1H), 5.48 (t, *J* = 3.1 Hz, 1H), 5.34−5.28 (m, 2H), 5.13−5.06 (m, 2H), 5.00 (dd, *J* = 3.1, 9.2 Hz, 1H), 4.75 (dd, *J* = 5.4, 9.6 Hz, 1H), 4.68 (d, *J* = 1.6 Hz, 1H), 4.62 (dd, *J* = 9.5, 11.5 Hz, 1H), 4.58 (ddd, *J* = 1.6, 4.6, 9.0 Hz, 1H), 4.28−4.20 (m, 2H), 4.08 (dd, *J* = 2.4, 12.2 Hz, 1H), 3.98−3.90 (m, 1H), 3.76 (ddd, *J* = 7.0, 8.5, 14.1 Hz, 1H), 3.40 (s, 3H), 2.22 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 170.7, 169.8, 169.8, 169.7, 169.1, 168.9, 168.7, 158.1, 157.3, 99.7, 72.3, 69.2,

68.0, 67.3, 67.1, 66.0, 63.7, 62.6, 60.3, 55.2, 52.4, 50.4, 39.7, 33.8, 20.7 (2 carbons), 20.7 (2 carbons), 20.6 (3 carbons); IR (neat) 3346 (w), 1750 (s), 1679 (m), 1223 (s), 1052 (m) cm^{-1}; MS (EI) *m/z* (rel int) 742 (M − OMe, <1), 654 (1), 538 (5), 455 (6), 113 (29), 55 (39), 43 (100); HRMS (ES) calcd for $C_{32}H_{43}N_3NaO_{19}$ (M + Na) 796.2388, found 796.2394.

(5*R*,6*R*,7*S*,8*S*,8a*S*)-*N*-(6,7,8-Triacetoxy-5-acetoxymethyl-1-{[1′*S*,2′*S*,3′*R*,4′*S*,5′*R*]-3′,4′-diacetoxy-5′-acetoxymethyl-1′-methoxytetrahydropyran-2′-ylbutyr-amide-4″-yl})-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione (45). 20% palladium hydroxide on carbon (15 mg) was added to a solution of benzyl ether **30g** (30 mg, 0.040 mmol) and cyclohexene (0.15 mL) in EtOH (0.60 mL) and refluxed with stirring for 2 h. At this point, analysis of the reaction by LCMS indicated complete removal of the benzyl ether so it was cooled to room temperature, filtered through Celite and concentrated to obtain a clear oil. The residue was dissolved in dry MeOH (0.8 mL), and ethylene glycol (11 μL, 12 mg, 0.20 mmol) and *p*-toluenesulfonic acid (8 mg, 0.04 mmol) were added. The reaction was stirred at reflux for 9 h at which point analysis by LCMS indicated that all of the acetonides had been removed. Therefore, the reaction was cooled to room temperature, quenched with pyridine (20 μL) and concentrated to give a pale yellow oil. The oil was dissolved in pyridine (0.4 mL) and acetic anhydride (0.4 mL) and stirred at room temperature for 16 h. The reaction was then concentrated to yield a pale yellow oil. Normal phase HPLC (100% EtOAc, 10 mL/min, t_R = 38.0 min) afforded 12 mg (39%) of the title compound as a clear oil. Data for **45**: R_f = 0.22 (100% EtOAc); $[\alpha]^{23}_D$ = 13.2° (*c* 0.60, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.45 (d, *J* = 9.0 Hz, 1H), 5.49 (t, *J* = 3.4 Hz, 1H), 5.30 (dd, *J* = 4.6, 10.2 Hz, 1H), 5.26 (d, *J* = 9.2 Hz, 1H), 5.10 (t, *J* = 10.2 Hz, 1H), 5.07 (d, *J* = 3.6 Hz, 1H), 4.95 (dd, *J* = 3.2, 9.3 Hz, 1H), 4.79−4.72 (m, 2H), 4.70 (d, *J* = 1.0 Hz, 1H), 4.56 (ddd, *J* = 1.4, 4.5, 9.0 Hz, 1H), 4.26−4.19 (m, 2H), 4.08−4.01 (m, 2H), 3.96 (ddd, *J* = 2.4, 5.9, 10.0 Hz, 1H), 3.41 (s, 3H), 3.34 (dt, 4.8, 14.2 Hz, 1H), 2.36−2.17 (m, 3H), 2.22 (s, 3H), 2.11 (app d, *J* = 3.9 Hz, 6H), 2.10 (app d, *J* = 3.1 Hz, 6H), 2.03 (s, 3H), 2.01−1.93 (m, 1H), 1.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.9, 171.8, 170.6, 169.8, 169.7, 168.9, 168.8, 168.6, 158.2, 157.1, 99.9, 72.4, 69.3, 67.9, 67.2, 66.2, 62.7, 62.4, 60.3, 55.2, 52.7, 50.2, 42.1, 33.3, 29.7, 23.5, 20.7 (3 carbons), 20.6 (3 carbons), 20.6; IR (neat) 3358 (w), 1750 (s), 1680 (m), 1224 (s), 1051 (s) cm^{-1}; MS (EI) *m/z* (rel int) 788 (M + H, 1), 756 (4), 527 (18), 469 (22), 242 (14), 84 (15), 43 (100); HRMS (ES) calcd for $C_{33}H_{45}N_3NaO_{19}$ (M + Na) 810.2545, found 810.2548.

(5*R*,6*R*,7*S*,8*S*,8a*S*)-*N*-(6,7,8-Trihydroxy-5-hydroxymethyl-1-{[1′*S*,2′*S*,3′*R*,4′*S*,5′*R*]-3′,4′-dihydroxy-5′-hydroxymethyl-1′-methoxytetrahydropyran-2′-ylacet-amide-2″-yl})-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione (7). Sodium methoxide powder (15 mg, 0.34 mmol) was added to a solution of compound **43** (26 mg, 0.034 mmol) in MeOH (0.7 mL) and stirred at room temperature for 30 min. The reaction was then passed over a short column of Dowex 50Wx8-100 resin (pyridinium form), the product was eluted with MeOH and concentrated to yield 13 mg (84%) of the title compound as a clear oil. Data for **7**: R_f = 0.01 (30% MeOH/CH_2Cl_2); $[\alpha]^{23}_D$ = 31.4° (*c* 0.67, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.18 (d, *J* = 9.2 Hz, 1H), 5.03 (d, *J* = 9.1 Hz, 1H), 4.63 (d, *J* = 1.1 Hz, 1H), 4.56 (ABq, *J* = 16.7, Δν = 18.8 Hz, 2H), 4.43 (dd, *J* = 5.0, 8.7 Hz, 1H), 4.35 (dd, *J* = 1.1, 4.8 Hz, 1H), 4.03 (d, *J* = 2.9 Hz, 1H), 3.98 (dd, *J* = 8.7, 11.9 Hz, 1H), 3.93−3.89 (m, 2H), 3.82 (d, *J* = 3.3 Hz, 2H), 4.76−3.70 (m, 2H), 3.57 (t, *J* = 9.7 Hz, 1H), 3.52 (dt, *J* = 3.3, 9.7 Hz, 1H), 3.37 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 170.0, 161.1, 159.8, 101.6, 74.0, 73.4, 73.3, 70.8, 70.4, 68.4, 68.1, 62.3, 61.7, 60.2, 55.3, 54.4, 46.2; IR (MeOH) 1739 (vs), 1673 (m), 1063 (s) cm^{-1}; MS (EI) *m/z* (rel int) 429 (M − 2 H_2O, <1), 339 (5), 137 (31), 59 (100); HRMS (ES) calcd for $C_{17}H_{27}N_3NaO_{12}$ (M + Na) 488.1492, found 488.1482.

(5*R*,6*R*,7*S*,8*S*,8a*S*)-1-(6′-Ammoniohexyl)-6,7,8-trihydroxy-5-hydroxymethyl-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione Chloride (47). A solution of **46** (44 mg, 0.085 mmol) in THF (0.85 mL) was treated with 1 N HCl (0.85 mL) and stirred at room temperature for 4 h. The reaction was then concentrated to yield 31 mg (98%) of the title compound as a yellow oil. The crude material was sufficiently pure for characterization. Data for **47**: R_f = 0.00 (10% MeOH/CH_2Cl_2); $[\alpha]^{23}_D$ = 22.3° (*c* 0.92, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.78 (s, 1H), 4.99 (d, *J* = 8.8 Hz, 1H), 4.40 (dd, *J* = 4.6, 8.8 Hz, 2H), 4.02 (d, *J* = 3.4 Hz, 1H), 3.98 (dd, *J* = 9.0, 11.7 Hz, 1H), 3.92 (t, *J* = 3.1 Hz, 1H), 3.83−3.70 (m, 3H), 3.67 (dd, *J* = 3.1, 9.0 Hz, 1H), 2.97−2.90 (m, 2H), 1.86−1.64 (m, 4H), 1.50−1.35 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 160.9, 160.5, 73.6, 73.6, 70.5, 67.8, 61.8, 60.2, 44.1, 40.8, 28.5, 28.5, 27.3, 26.9; IR (MeOH) 1730 (s) cm^{-1}; MS (ES) *m/z* (rel int) 332 (M + H, 100); HRMS (ES) calcd for $C_{14}H_{26}N_3O_6$ (M + H) 332.1822, found 332.1819.

(5*R*,6*R*,7*S*,8*S*,8a*S*)-1-(6′-Acetylaminohexyl)-6,7,8-trihydroxy-5-hydroxymethyl-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione (49). Compound **48** (24 mg, 0.044 mmol) was treated with 2 N NH_3−MeOH (0.90 mL, 1.8 mmol NH_3) and stirred at 40 °C for 1 h. The reaction was then concentrated to yield a clear oil. Chromatography (20% MeOH/CH_2Cl_2) afforded 11 mg (64%) of the title compound as a clear oil. Data for **49**: R_f = 0.07 (20% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.97 (d, *J* = 9.2 Hz, 1H), 4.40 (dd, *J* = 5.0, 8.6 Hz, 1H), 4.01 (dd, *J* = 1.1, 3.6 Hz, 1H), 3.97 (dd, *J* = 9.0, 11.9 Hz, 1H), 3.91 (t, *J* = 3.1 Hz, 1H), 3.86−3.68 (m, 1H), 3.66 (dd, *J* = 3.3, 9.2 Hz, 1H), 3.15 (t, *J* = 7.0 Hz, 2H), 1.91 (s, 3H), 1.84−1.64 (m, 2H), 1.55−1.45 (m, 2H), 1.43−1.32 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.2, 160.5, 160.3, 73.6, 73.5, 73.4, 67.6, 67.6, 61.7, 60.0, 44.1, 40.3, 30.2, 28.6, 27.4, 22.5; IR (neat) 3296 (br m), 1732 (s) cm^{-1}; MS (EI) *m/z* (rel int) 373 (M^+, 2), 355 (9), 337 (40), 306 (100); HRMS (ES) calcd for $C_{16}H_{27}N_3NaO_7$ (M + Na) 396.1747, found 396.1746.

Anhydrous Coupling of (5*R*,6*R*,7*S*,8*S*,8a*S*)-1-(6′-Ammoniohexyl)-6,7,8-trihydroxy-5-hydroxymethyl-hexahydroimidazo[1,2-*a*]pyridine-2,3-dione Chloride (47) to Affi-Gel 10 (10). Compound **10** was prepared using the anhydrous coupling protocol of Affi-Gel 10 (Bio-Rad) described in the manufacturer's manual.[48] A solution of the hydrochloride salt **47** (51 mg, 0.14 mmol) and triethylamine (0.5 mL) in MeOH (5 mL) was added to a slurry of Affi-Gel 10 resin (8.30 mL settled resin volume, 0.125 mmol, prewashed with 6 bed volumes of cold *i*PrOH and 6 bed volumes of cold MeOH) in MeOH (20 mL) and agitated on a shaker at 4 °C for 16 h. The gel was filtered and washed with cold MeOH (6 bed volumes) and cold water (6 bed volumes), and the combined filtrates were concentrated. The amount of **47** in the combined filtrates was determined to be approximately 0.013 mmol by ^{1}H NMR using dimethyl acetemide as an internal standard, corresponding to a loading of 0.015 mmol **47** per mL of gel. The gel was added to a solution of ethanolamine (6.25 mL, 0.1 M solution in water, 0.625 mmol) in cold water (25 mL) and agitated on a shaker at 4 °C for 4 h. The gel was then filtered and washed with cold water (6 bed volumes) and cold 0.2% aqueous NaN_3 (3 bed volumes) and stored at 0 °C in a solution of 0.2% aqueous NaN_3 (inhibits bacterial growth).

Assay of α1,2-Mannosidase Inhibition. The inhibitory activity of each compound was assayed at three concentrations (0.05, 0.5, and 5 mM) using recombinant human ER mannosidase I[5b] or recombinant mouse Golgi Man IA.[5b] ER Man I assays were performed using 20 μg of enzyme and $Man_9GlcNAc_2$-PA (20 μM) as substrate in a final volume of 20 μL containing 20 mM MES/NaOH, pH 7.0, 150 mM NaCl, and 5 mM $CaCl_2$. The enzyme reactions were allowed to proceed for 15 min at 37 °C and were stopped by addition of 20 μL of 1.25 M Tris-HCl pH 7.6. The human ER Man I product, $Man_8GlcNAc_2$-PA, was resolved from $Man_9GlcNAc_2$-PA by HPLC on a Hypersil APS-2 NH_2 column.[50] $Man_9GlcNAc_2$-PA was pre-

(50) Lal, A.; Pang, P.; Kalelkar, S.; Romero, P. A.; Herscovics, A.; Moremen, K. W. *Glycobiology* **1998**, *8*, 981−995.

pared by reductive pyridylamination of $Man_9GlcNAc_2$ isolated from crude soybean agglutinin extracted as described previously.[15b] Golgi Man IA assays were performed using 10 μg of enzyme in a reaction containing 20 mM potassium phosphate, pH 6.0, 150 mM NaCl, 5 mM $CaCl_2$, and 5 mM Man-α-1,2-Man-α-O-CH_3 in a total volume of 25 μL. Enzyme reactions were allowed to proceed for 30 min at 37 °C and terminated by the addition of 25 μL of 1.25 M Tris-HCl, pH 7.6. The amount of mannose released was quantified using a glucose oxidase and peroxidase reagent as previously described.[50] The recombinant enzymes were expressed and purified as previously described.[5b] For determination of compound inhibition, enzyme activity was plotted as a percentage of residual activity (compared to no added compound) versus compound concentration and the IC_{50} values were estimated from the plots.

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Supporting Information Available: Experimental procedures for compounds **2**−**6**, **8**, **9**, **12**, **19**, **20**, **28b**, **28c**, **30a**−**c**, **30e**−**g**, **32**−**35**, **40**−**42**, **46**, and **48** and ^{1}H spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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