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ARTICLE

Investigations into the decomposition of aminoacyl-substituted monosaccharide scaffolds from a drug discovery library

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This study investigated the unexpected decomposition and associated intermediates of compound **1**, a specific member of a drug discovery library based on a monosaccharide scaffold. LC/MS and NMR spectroscopic analyses indicated that, under acidic conditions, **1** can be converted into the 4-aminogalactoside **2**, due to cleavage of the 4-aminobutanoyl side chain. The reaction occurs most likely through an initial intramolecular amino-amide interaction, followed by an *N*- to *O*-acyl transfer of the side chain from C-4 to the C-6 position to form an ester intermediate (**5**), detectable by NMR, and subsequent hydrolysis. Similar decomposition reactions could be induced in selected compounds with similar structures, containing a free hydroxyl group at C-6 and a 4-aminobutanoyl side chain at C-4 of an aminogalactoside. Furthermore, three model compounds were synthesized without a C-6 hydroxyl group and with different length aminoalkanoyl side chains at the C-4 position. The model compounds all decomposed under acidic conditions, but at different rates and much slower when compared with compound **1**, suggesting that both the C-6 hydroxyl group and the length of the side chain have an influence on stability.

Introduction

Monosaccharide scaffolds provide an excellent platform for drug discovery with the aim of accurately transposing known peptide ligands onto a metabolically stable scaffold while maintaining a good level of bioactivity.[1-4] For example, in the past two decades such scaffolds have been successfully used to convert bioactive peptides into more drug-like molecules.[2,5-7] A number of unique features of monosaccharide scaffolds contribute to the generation of diverse chemical libraries.[4,8] For instance, with only one scaffold and three different substituents, there are already 60 different ways to present these substituents to a binding site.[4,8] This increases to almost 96 different presentations by changing just one of the substituted chiral centres.[4,8] Therefore, compounds built on monosaccharide-based templates can rapidly attain high molecular diversity within one class of compound, as well as leading to rigid products with well-defined geometry.[1,2] This could increase hit rates and help to efficiently identify lead compounds for potential therapeutic targets from the structurally related sets of hits.[1,2]

In efforts to obtain systematic diverse libraries using monosaccharide scaffolds, a solid phase synthesis technology has been developed [Versatile Assembly on Sugar-like Templates (VAST^{TM})] enabling parallel and efficient generation of structurally diverse libraries.[3,9] Three orthogonally protected monosaccharide scaffolds, based on D-galactopyranose, D-glucopyranose and D-allopyranose were identified.[10,11] These three scaffolds were designed to include a resin attachment point and utilised protecting groups that are stable to various reaction conditions required to introduce the substituents.[10] In addition, these scaffolds could be readily prepared in good yield at large scale ranging from 100 g to > 1 kg.[10] These scaffolds have been successfully used as the starting points in the preparation of systematic diverse libraries via solid phase synthesis.[10] This allows the main reactions for library preparation to be carried out on resin, simplifying the manipulation of the library,[4,9] and thus providing a platform for efficient synthesis of compounds with high molecular diversity for pre-clinical assessment.

The galactoside derivative **1** (Fig. 1) was synthesized as part of a diversity scanning library (DSL) production process.[9-11] After approximately 2.5 years of storage at 2–8 °C (10 mM in DMSO), this sample was identified as a hit against a proprietary drug target following a high-throughput screening campaign. However, LC/MS analysis showed that the sample had decomposed to a compound with MW = 445 (Fig. S1, t_R = 4.79 min, *m/z* 446.22 $[M+H]^+$). Thus, the decomposition product was suspected to be responsible for the observed activity. Herein we report the results of investigations into the structure, stability and decomposition of compound **1** and some related compounds, the structural elucidation of the decomposition product and a key intermediate, and propose a mechanism for their formation.

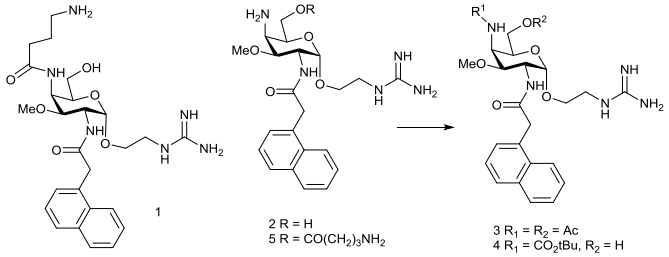


Figure 1. The structure of compound **1**, decomposition product **2**, derivatives **3** and **4**, and proposed intermediate **5**.

Results and Discussion

Investigation of the structure of the decomposition product

Due to the limited amount of the decomposition product available for further study, compound **1** was resynthesized[9-11] at larger scale (m = 510 mg) and was fully characterized by NMR spectroscopy in both DMSO-d_6 and MeOH-d_4, and by LC/MS (Fig. S2, t_R = 4.87 min, *m/z* 531.10 $[M+H]^+$). All ^1H and ^{13}C NMR chemical shifts were fully assigned using a combination of COSY, HSQC and HMBC experiments (see Supporting Information, Tables S1-2). The newly synthesized sample of **1** was inactive in the bioassay. Tests were then conducted on compound **1** under various conditions in order to identify the optimal conditions to reproduce the originally observed decomposition. Interestingly, when **1** was heated in DMSO solution (10 mM, to reproduce the storage conditions) containing 5% H_2O in the presence of TFA (10%, pH 1) at 50 °C, after 2 days, a more polar intermediate with mass 530 was observed by LC/MS (Fig. S3b, t_R = 4.38 min, *m/z* 531 $[M+H]^+$, ~ 5%). After 6 days, a product with mass 445 had started to form slowly (data not shown). Heating was continued for 13 days and then the sample was left at room temperature. After 13 days heating, the amount of the intermediate had increased significantly (Fig. S3c, t_R = 4.37 min, *m/z* 531 $[M+H]^+$, ~ 30%). After 49 days, the more polar intermediate was no longer detectable (data not shown). After 10 months, only a single product could be detected by LC/MS (Fig. S3d, t_R = 5.11 min, *m/z* 446 $[M+H]^+$), which could possibly be the same as the decomposition product initially observed from the DSL.

In an attempt to more rapidly reproduce the decomposition product, compound **1** (1 mM) was treated with 10% TFA in H_2O (pH 1) at 80 °C. After one hour, a more polar intermediate with the same mass as the starting material was detected by LC/MS (Fig. 2a, t_R = 4.47 min, *m/z* 531 $[M+H]^+$, ~ 10%). After 13 hours, the starting material was totally decomposed to another compound with mass 445 (Fig. 2b, t_R = 4.80, *m/z* 446 $[M+H]^+$). HPLC co-elution experiments showed that the resynthesized decomposition product and the one observed from the DSL were co-eluted when injected at similar concentrations (data not shown), hence, it was concluded that the resynthesized decomposition product was likely to be the same as the one observed in the original DSL sample. When **1** was treated with 10% TFA in DMSO, there was more intermediate formed; however, the formation of the final decomposition product was much slower than when the reaction was performed in water.

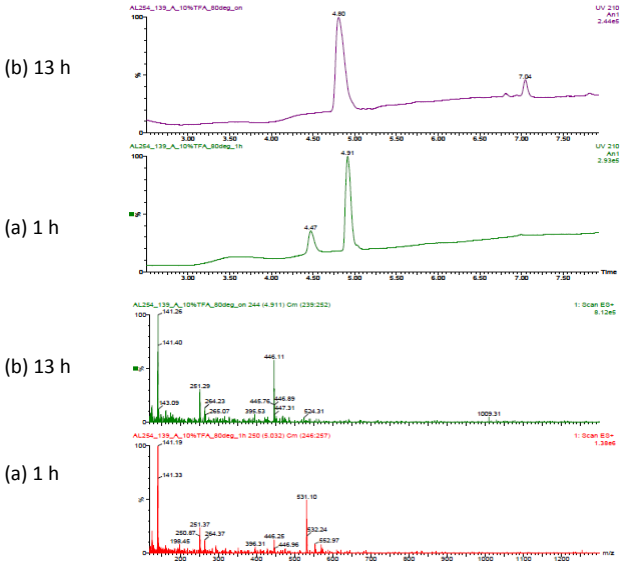


Figure 2. LC/MS analysis of **1** (1 mM) in H_2O with 10% TFA, pH 1 and 80 °C: (a) after 1 h; (b) after 13 h.

In order to identify the structure of the decomposition product, another sample of **1** was treated with aqueous 10% TFA at 80 °C as described above. After 10 minutes, the more polar intermediate was observed by LC/MS and after 20 hours, the decomposition product had formed (t_R = 4.91 min, *m/z* 446.58 $[M+H]^+$). After evaporation of the solvent, the crude residue was purified by passage through a reverse phase SPE C_{18} cartridge to afford the product in 94% yield. The resynthesized decomposition product was fully characterized by NMR spectroscopy in two different solvents, DMSO-d_6 and MeOH-d_4. All ^1H and ^{13}C chemical shifts were fully assigned using a combination of COSY, HSQC and HMBC experiments (see Supporting Information, Tables S3-4). In comparison with the NMR spectra of **1**, the NMR spectra of the decomposition product revealed that the peaks for the 4-aminobutanoyl side chain were no longer present. In addition, the proton assigned to H-4 was shifted upfield from 4.55 ppm to 3.58 ppm, consistent with the loss of the amide group. Moreover, the NMR signals for the other protons had similar chemical shifts in all respects to that of **1**. The NMR data was thus consistent with 4-aminogalactoside **2** as the decomposition product. These NMR data were initially surprising because compound **2** is a member of the DSL and was inactive in the original high-throughput screening assay.

Further confirmation of the structure of the decomposition product was obtained by the preparation of two derivatives. Firstly, treatment with excess acetic anhydride and a catalytic amount of DMAP in pyridine at 50 °C for 1.5 hours followed by purification of the reaction mixture by reverse phase SPE C_{18} cartridge gave the diacetate **3** (t_R = 5.80 min, *m/z* 530.47 $[M+H]^+$). A tetraacetate derivative was also detected by LC/MS of the reaction mixture (t_R = 6.44 min, *m/z* 614.51 $[M+H]^+$, 636.40 $[M+Na]^+$), presumably due to acetylation of the guanidino group, however, this product was unstable and could not be isolated. Treatment of the decomposition product with di-*tert*-butyldicarbonate and triethylamine in anhydrous

DMF at room temperature for 65 minutes followed by purification by reverse phase SPE C_{18} cartridge gave a single product, the carbamate **4** (t_R = 6.45 min, *m/z* 546.59 $[M+H]^+$). The ^1H NMR and COSY spectra of **3** and **4** showed the expected downfield shifts for H-4 (4.61 and 4.18 ppm, respectively) and H-6 (for **3**, 4.02 ppm). Finally, an authentic sample of **2** from the DSL (t_R = 4.92 min, *m/z* 446.58 $[M+H]^+$) was re-purified by reversed phase SPE C_{18} cartridge and analyzed by ^1H NMR spectroscopy, which showed that the two samples were identical. The data confirm that the final decomposition product was the 4-aminogalactoside **2**, however, the nature of the initially formed intermediate and the identity of the bioactive compound were unclear.

Investigation of the structure of the reaction intermediate

In order to confirm that the observed reaction intermediate could be converted into **2** under acidic conditions (pH 1), a small amount of the intermediate (50 μg) was isolated by HPLC from the reaction of **1** (1 mg) in 10% TFA in H_2O after 10 minutes at 80 °C (t_R = 3.97 min, *m/z* 531 $[M+H]^+$). The isolated intermediate was then treated with 10% TFA in H_2O (300 μL, pH 1) for two days at room temperature and the reaction monitored by LC/MS. LC/MS analysis showed that the isolated intermediate was slowly converted into **2** at room temperature under these conditions (t_R = 4.88 min, *m/z* 446 $[M+H]^+$). The intermediate was then isolated on a larger scale (2 mg) by HPLC from the reaction of **1** (20 mg) with 10% TFA in H_2O (pH 1) after 10 minutes at 80 °C (t_R = 4.09, *m/z* 531 $[M+H]^+$). The sample was dissolved in MeOH-d_4 (pH 7) and analyzed by ^1H NMR and COSY spectroscopy. However, the NMR spectra indicated that the intermediate had been converted back into the starting material **1** under neutral conditions. This was further confirmed by LC/MS analysis of the NMR sample (t_R = 4.97, *m/z* 531 $[M+H]^+$).

Given that the reaction intermediate was unstable under neutral conditions and was difficult to isolate on a large scale by HPLC, an NMR experiment was performed. A sample of **1** (6.5 mg) in a mixture of 10% TFA in anhydrous DMSO-d_6 (pH 1) was heated at 80 °C for 6 hours in an NMR tube. The reaction progress was monitored by ^1H NMR and COSY spectroscopy (see Supporting Information). The NMR spectra showed that the ratio between **1** and the intermediate was approximately 3: 2 after 6 hours heating and no product **2** was formed in the absence of water. The NMR data was analyzed and a possible structure of the reaction intermediate was postulated from these data as the 6-*O*-ester **5** (Figure 1).

Compared with the ^1H NMR spectrum of **1**, in the reaction mixture it was noteworthy that H-4 of the intermediate was shifted upfield from 4.56 ppm to 3.88 ppm, while H-5 and H-6a,b, respectively, were shifted downfield (3.88 to 4.27 ppm, and 3.31 to 4.13 ppm, respectively). These shifts are consistent with migration of the 4-aminobutanoyl side chain from C-4 to C-6. In addition, the signal for the amide proton attached to C-4 was shifted slightly downfield to 8.03 ppm and integrated for two protons, suggesting the formation of an amino group. Furthermore, no cross peak was observed in the HMBC spectrum between this signal and the carbonyl group of the 4-aminobutanoyl side chain. Moreover, a ^{13}C – ^1H long range interaction between protons H-6a,b of the intermediate and the carbonyl group was detected by HMBC, confirming the presence of the 4-aminobutanoyl group at the C-6 position. Taken together, these NMR data were consistent with the proposed structure **5**. To further support the presence of an ester group at C-6, the reaction mixture containing **1** and the intermediate (3:2) was treated with NaOMe (pH 11) at 50 °C for 10 minutes. LC/MS analysis showed that **1** was stable to these conditions, while the intermediate was readily converted into 4-aminogalactoside **2** presumably due to transesterification of the aminobutanoyl side chain.

A proposed reaction mechanism for the formation of the product **5** from **1** under acidic conditions is shown in Figure 3. The reaction may proceed via an initial intramolecular amino-amide interaction[12-14] to give a cyclic hemi-orthoamide intermediate. Such intermediates have been previously isolated and characterized by NMR spectroscopy[15] but are known to be unstable and rapidly decompose with migration of the acyl group from *N* to *N*'.[16] Such an acyl migration pathway could account for loss of the 4-aminobutanoyl side chain via formation of butyrolactam. However, to account for formation of ester **5** we propose that the hemi-orthoamide undergoes dehydration followed by intramolecular nucleophilic attack from the C-6 OH and proton transfer to give an intermediate which then breaks down with migration of the side chain to O-6. Hydrolysis of this intermediate then generates the ester **5**. The product **2** could then be formed by simple hydrolysis of the ester or by an intramolecular attack by the γ-amino group to form butyrolactam and **2**. We cannot rule out the possibility of direct *N*- to *O*-acyl transfer by attack of the C6-OH on the amide carbonyl to form a cyclic hemi-orthoamide which decomposes with transfer of the side chain to C6. Such *N*- to *O*-acyl transfers have been reported for β,γ-bishydroxy amides under acidic conditions and have been postulated to proceed via a five-membered ring hydroxyoxazolidine intermediate.[17] Similarly, cyclosporins are known to undergo *N*- to *O*-acyl transfers to form isocyclosporins.[18] However, both these examples do not contain an additional amino group on the acyl side chain while our additional studies of related compounds with different length aminoacyl side chains (see below) indicate that overall stability is dependent on the length of the side chain. Unlike the well-known *O*- to *N*-acyl and *O*- to *S*-acyl transfers,[19-21] *N*- to *O*-acyl transfers as described above are relatively uncommon and presumably the D-galactoside scaffold positions the C-6 hydroxyl group in a favourable orientation to facilitate this process.

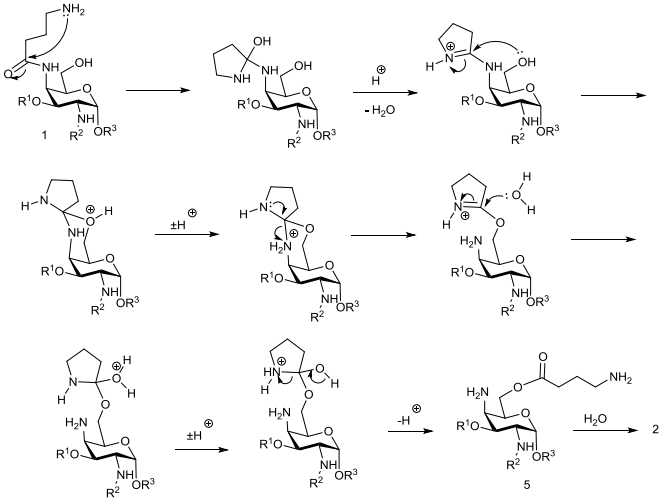


Figure 3. Proposed reaction mechanism for the decomposition of **1**. $R^1 = CH_3$, $R^2 = COCH_2Ar$, $R^3 = CH_2CH_2NHC(=NH)NH_2$.

Quality control of similar DSL compounds

Available compounds from the DSL (**6**-**10**, Fig. 4) with similar structures to **1** were next investigated to determine if a similar decomposition could occur for these samples. Compounds **6**-**9** were thus treated with 10% TFA in H_2O (pH 1) at 80 °C overnight and the reactions were analyzed by LC/MS. Compounds **6**-**8**, which contained a 4-aminobutanoyl side chain at C-4, were all susceptible to decomposition and gave final products corresponding to loss of the side chain. This was confirmed via a co-elution LC/MS experiment of the decomposition product of **6** with compound **10**, also available from the DSL, which showed that both compounds eluted at t_R = 6.87 minutes. No polar intermediate was observed during the reaction of **6**, possibly because the rate of reaction was too fast and not detectable by LC/MS. However, for both compounds **7** and **8** a more polar intermediate with the same mass as the starting material was observed after 20 minutes and one hour, respectively (t_R = 4.75, *m/z* = 559 $[M+H]^+$; t_R = 3.99, *m/z* = 511 $[M+Na]^+$), before loss of the aminobutanoyl side chain after overnight reaction. On the other hand, compound **9** with an aminoacetyl side-chain was completely stable to the reaction conditions. These results suggest that compounds with a 4-aminobutanoyl side chain undergo a similar decomposition reaction to **1** under acidic conditions, but with slight variations in the reaction rates, probably arising from the slight differences in their structures.

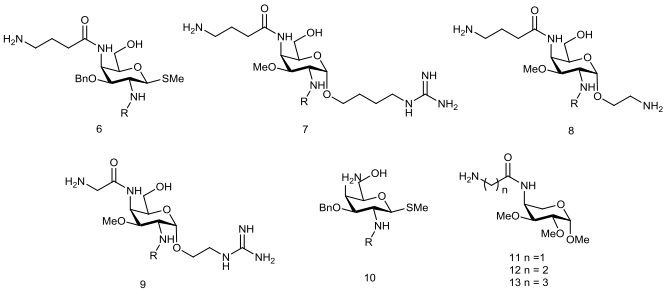
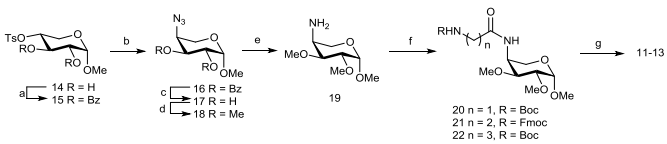


Figure 4. DSL compounds **6**-**10** with similar structures to **1** (R = $COCH_2Ar$), and model compounds **11**-**13**.

Model compound studies

Some simple model compounds (**11**-**13**) were next synthesized in order to further investigate the effects of the length of the side chain on stability, and to determine if the C-6 hydroxyl group was critical to the degradation process. The synthesis of **11**-**13** required the preparation of the common intermediate amine **19** from methyl 4-*O*-tosyl–α-D-xylopyranoside **14** (Scheme 1).



Scheme 1. Reaction scheme for the synthesis of model compounds **11**-**13**. *Reagents and conditions*: (a) BzCl, py, 0 °C→r.t., 18 h, 96%; (b) NaN_3, DMF, 90 °C, 48 h, 81%; (c) NaOMe, MeOH, 88%; (d) NaH, MeI, DMF, 93%; (e) H_2, 10% Pd/C, MeOH, 91%; (f) HBTU, DIPEA, DMF, Boc- or Fmoc-protected amino acid, 13-32%; (g) 1:2:7 TFA/Et_3SiH/CH_2Cl_2 (for Boc); and ethylenediamine resin/ piperidine/ DMF (for Fmoc), 91-95%.

Commercially available methyl α-D-xylopyranoside was regioselectively tosylated via its stannylene acetal following the procedure of Kosma[22] to give a 3:1 mixture of 4-*O*- and 2-*O*-tosylates from which 4-*O*-tosylate[23] **14** was readily isolated by flash chromatography. Tosylate **14** was then benzoylated with benzoyl chloride in pyridine at room temperature to give the dibenzoate[24,25] **15** in 96% yield. The melting point was slightly lower than previously reported, presumably due to the presence of trace impurities (visible in the ^1H NMR spectrum). Interestingly, in the ^1H NMR spectrum of **15** the signal for H-3 at 5.88 ppm appeared as a second order multiplet (apparent triplet of triplets) due to virtual coupling[26] to H-1 (δ H-1−δ H-2 = 5.0 Hz; $J_{1,2}$ = 3.5 Hz). The tosylate was then displaced with inversion of configuration by heating with sodium azide in DMF to give the azide[24] **16** in good yield (81%). Transesterification of the benzoate groups gave the diol[24] **17** in 88% yield which was then methylated with sodium hydride and methyl iodide in anhydrous DMF to give **18** in 93% yield. Reduction of the azido group was accomplished by hydrogenation in MeOH with 10% Pd/C as catalyst to furnish the amine **19** in 91% yield. The structure of **19** was supported by ^1H NMR spectroscopy (H-4 was shifted significantly upfield to 3.30 ppm) and LRMS (*m/z* 192.0 $[M+H]^+$), however, **19** was somewhat unstable, and so was reacted immediately in the next step without further purification or characterization. Amine **19** was coupled with (Boc-γ-amino)butyric acid, Fmoc-β-Ala-OH and Boc-Gly-OH, respectively, using HBTU and Hünig's base in anhydrous DMF at room temperature to give the carbamates **20**-**22**. The carbamates were obtained in unoptimised yields of 13, 32 and 19 %, respectively, following purification by flash chromatography, and were fully characterized. The target amines **11**-**13** were then obtained in excellent yield (91-95%) by removal of the Boc and Fmoc protecting groups via treatment with 1:2:7 trifluoroacetic acid/triethylsilane/dry dichloromethane (for Boc) and polymer supported ethylenediamine resin and piperidine in anhydrous DMF (for Fmoc), respectively.

Stability of model compounds under acidic conditions

The model compounds **11-13** were treated with 10% TFA in H_2O (pH 1) at 80 °C overnight to investigate if their stability was influenced by the length of the side chain at C-4 through an intramolecular amino-amide interaction. Unfortunately, the compounds were too polar for satisfactory analysis by LC/MS, so reaction progress was analyzed by TLC (*tert*-butanol/ acetic acid/ water, 2:1:1) and ^{1}H NMR and COSY spectroscopy. No intermediates were observed during the reaction via these techniques, only starting materials and products. In addition, the reaction rates were much slower than for the DSL compounds, with starting material still present after overnight reaction. ^{1}H NMR spectroscopic analysis after overnight reaction confirmed the major decomposition product to be amine **19** in all cases but the reaction rate for each compound was different. By comparison of the integral of the peaks for H-4 for the starting material and for the product amine **19** (at 3.64 ppm), it was possible to establish the relative stability of the three model compounds. The relative ratio of starting material to **19** increased with increasing chain length from 1:0.4 for **11**, to 1:0.7 for **12** and 1:0.8 for **13**. Whilst no intermediates were detected, we hypothesize that compounds **12** and **13** are more labile because they can form cyclic hemi-orthoamide intermediates[15] via intramolecular amino-amide interactions, whilst **11** cannot. The results also suggest that decomposition is less facile without the presence of a free hydroxyl group at C-6 to form an ester intermediate, but further studies are needed to confirm this.

Conclusions

In conclusion, the decomposition of compound **1** occurred during long term storage in DMSO (10 mM) to form the 4-aminogalactoside **2**, most likely via a mechanism involving migration of the side chain from C-4 to C-6 to form an ester intermediate (**5**) followed by hydrolysis to furnish compound **2**. Selected compounds from the DSL with similar structures to **1** are also capable of undergoing a similar decomposition reaction under acidic conditions with formation of an intermediate. Furthermore, three model compounds **11-13** with different length side chains at C-4 and without a C-6 hydroxyl group were also decomposed with different reaction rates under acidic conditions. It can be concluded that the stability 4-aminoacyl-D-galactoside derivatives is related to the length of the side chain with a 4-aminobutanoyl side chain being the most labile. In addition, the presence of a free hydroxyl group at C-6 appears to accelerate the rate of decomposition and results in an unusual *N*- to *O*-acyl transfer to give an ester intermediate. The nature of the bioactive species in the drug screening assay is still unclear and remains of ongoing interest.

Experimental

General. Compound **1** and similar compounds from the DSL were obtained from Alchemia Ltd. All reagents and solvents were obtained from Sigma-Aldrich (Australia) and were used without further purification, except MeOH, dichloromethane, EtOAc, hexane and EtOH which were distilled prior to use. 1D and 2D NMR spectra were recorded on a Bruker spectrometer (^{1}H NMR, COSY, HSQC and HMBC at 500 MHz, ^{13}C NMR at 100 MHz). The residual solvent peaks served as internal standard ($CDCl_3$: δ H 7.24 and δ C 77.0; DMSO-d_6: δ H 2.49 and δ C 39.5; MeOD: δ H 4.78 and δ C 49.0). Melting points were determined on a DigiMelt MSRS apparatus. High resolution mass spectra were recorded on a Bruker micrOTOF$_Q$ spectrometer in positive ion ESI mode. Chromatographic separations were carried out on silica gel (0.04-0.06 mm, 230-400 mesh, Merck) or a reverse phase SPE C_{18} cartridge. Reactions were monitored by reverse phase LC/MS or TLC, as appropriate. Reverse phase LC/MS was used under the following conditions: column: Zorbax SB-C_{18}, flow rate: 2 mL/min, time: 12 min, solvent A: 0.1% HCOOH in H_2O, solvent B: 0.1% HCOOH in MeCN. TLCs were visualized by staining/charring with ninhydrin (5% ninhydrin in ethanol), $KMnO_4$ (1.5 g $KMnO_4$, 10 g K_2CO_3 and 1.25 mL 10% NaOH in 200 mL water), or 5% sulfuric acid in ethanol.

Re-synthesis of the decomposition product (2)
Compound **1** (20 mg, 40 μmol) was dissolved in a mixture of 10% TFA in H_2O (36 mL, pH 1) and stirred at 80 °C for 20 h. The reaction was monitored by TLC (*n*-butanol/ H_2O/ HOAc, 26/25/6)[24] and LC/MS. After the reaction was complete, the resulting mixture was diluted with H_2O (50 mL), concentrated and co-evaporated with toluene (5 mL) to give the crude product. Purification by reverse phase SPE C_{18} cartridge (5% *i*-PrOH-H_2O + 0.1% HCOOH → 100% *i*-PrOH-H_2O + 0.1% HCOOH) gave compound **2** (16 mg, 94%) as white solid. R_f = 0.61 (*n*-butanol/ H_2O/ HOAc, 26/25/6); LC/MS: t_R = 4.91 min, *m/z* = 446.58 $[M+H]^+$, 468.47 $[M+Na]^+$; ^{1}H and ^{13}C NMR data are given in Tables S3-4.

Acetylation of compound 2. To a solution of compound **2** (8 mg, 18 μmol) in pyridine (0.5 mL) was added Ac_2O (47 μL, 0.50 mmol) and a catalytic amount of DMAP. The reaction mixture was sonicated at 50 °C under N_2 for 1.5 h. The reaction was monitored by LC/MS. The reaction mixture was cooled (0 °C) and anhydrous MeOH (2 mL) was added to remove the excess Ac_2O. The solution was stirred at 0 °C for 1 h and was then concentrated and co-evaporated with toluene (3 × 1 mL) to give the crude product as a light yellow solid. Purification by reverse phase SPE C_{18} cartridge (5% *i*-PrOH-H_2O + 0.1% HCOOH → 100% *i*-PrOH-H_2O + 0.1% HCOOH) gave the diacetate **3** (2 mg, 22 %) as white solid. LC/MS: t_R = 5.73 min, *m/z* = 530.62 $[M+H]^+$. ^{1}H NMR (500 MHz, MeOH-d_4): δ 7.96 (d, *J* = 8.0 Hz, 1H, H-24), 7.81 (m, 1H, H-21), 7.74 (m, 1H, H-19), 7.46-7.41 (m, 4H, H-23, H-22, H-18, H-17), 4.78 (1H, H-1, overlap with MeOD peak), 4.61 (dd, *J* = 1.5, 4.0 Hz, 1H, H-4), 4.16 (dd, *J* = 4.0, 11.5 Hz, 1H, H-2), 4.06-3.94 (m, 5H, H-5, H-6, H-15), 3.61 (m, 1H, H-7a), 3.50 (dd, *J* = 4.5, 11.5Hz, 1H, H-3), 3.44 (m, 1H, H-7b), 3.29 (m, 1H, H-8a), 3.25 (s, 3H, OCH_3), 3.18 (m, 1H, H-8b), 1.95 (s, 3H, CH_3), 1.93 (s, 3H, CH_3).

Synthesis of carbamate 4. To a mixture of **2** (5 mg, 11 μmol) and $(Boc)_2O$ (3.1 μL, 13 μmol, 1.2 eq.) in anhydrous DMF (1 mL) was added Et_3N to adjust the pH to 10. The mixture was sonicated at room temperature for 65 min, diluted with toluene and evaporated under reduced pressure to remove DMF. The crude product was purified by reverse phase SPE C_{18} cartridge (30% *i*-PrOH-H_2O + 0.1% HCOOH → 100% *i*-PrOH + 0.1% HCOOH) to give the carbamate **4** (3 mg, 50%) as white solid. LC/MS: t_R = 6.66 min, *m/z* = 546.59 $[M+H]^+$. ^{1}H NMR (500 MHz, MeOH-d_4): δ 7.97 (d, *J* = 8.5 Hz, 1H, H-24), 7.81 (d, *J* = 8.0Hz, 1H, H-21), 7.74 (dd, *J* = 2.0, 7.5Hz, 1H, H-19), 7.48 – 7.36 (m, 4H, H-23, H-22, H-18, H-17), 4.74 (d, *J* = 4.0Hz, 1H,

H-1), 4.18 (d, J = 3.0Hz, 1H, H-4), 4.08 (dd, J = 4.0, 11.5Hz, 1H, H-2), 3.97 (s, 2H, H-15), 3.82 (t, J = 5.0 Hz, 1H, H-5), 3.61 – 3.57 (m, 1H, H-7a), 3.51 – 3.50 (m, 2H, H-6), 3.45 (dd, J = 4.0, 11.5Hz, 1H, H-3), 3.40 – 3.36 (m, 1H, H-7b), 3.28 – 3.27 (m, 1H, H-8a), 3.25 (s, 3H, OCH_3), 3.20 – 3.16 (m, 1H, H-8b), 1.37 (s, 9H, CH_3).

Purification of 2 from DSL. Resin-bound **2** was treated with cleavage solution (TFA/ TES/ DCM, 1:2:7, 3 mL) under N_2 for 3 h at room temperature. The cleavage solution was then drained under N_2. The resin was then washed with MeCN (4 × 4 mL), a solution of MeCN with 10% H_2O (5 mL), and co-evaporated with toluene (2 × 2 mL) to afford the crude cleavage product. It was then purified by reversed phase SPE C_{18} cartridge (5% *i*-PrOH-H_2O + 0.1% HCOOH → 100% *i*-PrOH + 0.1% HCOOH) to give the product **2** (2 mg) as white solid. LC/MS: t_R = 4.76, m/z = 446.65 $[M+H]^+$. The ^{1}H NMR and COSY spectra of the product were identical in all respects to that prepared by decomposition of compound **1**.

Attempted isolation of the reaction intermediate. Compound **1** (20 mg, 40 μmol) was dissolved in a mixture of 10% TFA in H_2O (36 mL, pH1) and stirred at 80 °C for 10 min. The reaction was stopped by cooling the reaction mixture (dry ice/acetone bath). The solution was then purified by HPLC to give the intermediate as white solid (2 mg) after drying under high vacuum. It was then subjected to analysis by ^{1}H NMR and COSY spectroscopy. However, it was found that the product had been converted back into **1** under neutral conditions at room temperature, as judged by ^{1}H NMR, COSY and LC/MS which were identical in all respects to that of authentic **1**.

General experimental procedure for LC/MS experiments for the quality control of similar DSL compounds. DSL compounds **6**-**10** were first analysed by LC/MS. Samples (0.15 mg) were dissolved in a mixture of 10% TFA in H_2O (300 μL, pH 1). The resulting solutions were stirred at 80 °C overnight. The reaction progress was examined by LC/MS.
6: t_R = 6.58 min, m/z = 552.57 $[M+H]^+$, 574.60 $[M+Na]^+$; **7**: t_R = 5.21 min, m/z = 559.68 $[M+H]^+$; **8**: t_R = 4.50 min, m/z = 489.65 $[M+H]^+$, 511.61 $[M+Na]^+$; **9**: t_R = 5.15 min, m/z = 503.52 $[M+H]^+$, 525.41 $[M+Na]^+$; **10**: t_R = 6.55 min, m/z = 467.48 $[M+H]^+$. 489.51 $[M+Na]^+$.

Methyl 4-*O*-*p*-toluenesulfonyl-α-D-xylopyranoside (14). Methyl α-D-xylopyranoside was tosylated following the method of Kosma[22] to give a mixture of tosylates (1.0 g). Purification by flash chromatography (EtOAc/ toluene, 3:2) gave the 4-*O*-tosylate **14** (745 mg, 75%) and the 2-*O*-tosylate (250 mg, 25%). 4-*O*-tosylate **14**: R_f = 0.28 (EtOAc/ toluene, 3:2), colourless needles, m.p. 50-55 °C (EtOAc – hexane; lit.[28] 59-60 °C (benzene); lit.[29] 57-58 °C (benzene)); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84-7.78 (m, 2H, Ar), 7.35-7.32 (m, 2H, Ar), 4.67 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.32 (ddd, 1H, $J_{3,4}$ = 9.0 Hz, $J_{4,5a}$ = 5.8 Hz, $J_{4,5b}$ = 10.5 Hz, H-4), 3.80 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-3), 3.68 (dd, 1H, $J_{5a,5b}$ = 11.2 Hz, H-5a), 3.58 (dd, 1H, H-5b), 3.46 (dd, 1H, H-2), 3.38 (s, 3H, OCH_3), 2.43 (s, 3H, $ArCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.3, 133.0, 129.9, 128.0 (Ar), 98.8 (C-1), 77.9 (C-4), 72.3 (C-2), 72.0 (C-3), 58.8 (C-5), 55.6 (OCH_3), 21.7 ($ArCH_3$). 2-*O*-tosylate: R_f = 0.34 (EtOAc/ toluene, 3:2), colourless needles m.p.141–142 °C (EtOAc-hexane; lit.[23] 140–141 °C). ^{1}H NMR (500 MHz, CDCl3): δ 7.83-7.78 (m, 2H, Ar), 7.36-7.32 (m, 2H, Ar), 4.62 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.24 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 3.87 (ddd, 1H, $J_{3,OH}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.66 – 3.61 (m, 2H, H-4, H-5a), 3.47 (dd, 1H, $J_{4,5b}$ = $J_{5a,5b}$ = 11.5 Hz, H-5b), 3.26 (s, 3H, OCH_3), 2.68 (d, 1H, 3-OH), 2.52 (d, 1H, $J_{4,OH}$ = 3.0 Hz, 4-OH), 2.44 (s, 3H, $ArCH_3$); ^{13}C NMR (100 MHz, CDCl3): δ 145.3, 133.9, 129.9, 128.0 (Ar), 97.4 (C-1), 79.5 (C-2), 71.6 (C-3), 70.0 (C-4), 60.6 (C-5), 55.4 (OCH_3), 21.7 ($ArCH_3$).

Methyl 2,3-di-*O*-benzoyl-4-*O*-*p*-toluenesulfonyl-α-D-xylopyranoside (15). Benzoyl chloride (0.61 mL, 5.28 mmol) was added dropwise to a solution the 4-*O*-tosylate 14 (700 mg, 2.20 mmol) in anhydrous pyridine (8 mL) at 0 °C under N_2. The colourless solution turned yellow and a colourless solid precipitated. The ice bath was removed and the reaction mixture was stirred at room temperature for 18 h. The resulting mixture was dissolved in $CHCl_3$ (50 mL) and washed with H_2O (50 mL). The aqueous layer was extracted with $CHCl_3$ (40 mL), the organic layers were combined, washed with aq 0.1 M HCl (50 mL) and sat. aq $NaHCO_3$ (50 mL), and dried ($MgSO_4$). The solution was concentrated and the solid residue was crystallized from *n*-hexane-EtOAc to give the dibenzoate **15** as colourless needles (1.11 g, 96%), R_f = 0.60 (toluene/EtOAc, 4:1), m.p. 177.5 – 178.6 °C (lit.[24] 180 – 183 °C; lit.[25] 185 – 186 °C); The observed melting point was low due to the presence of traces of impurities (~ 3%), as seen in the ^{1}H NMR spectrum. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88-7.85 (m, 2H, Ar), 7.68-7.65 (m, 2H, Ar), 7.62-7.57 (m, 2H, Ar), 7.49 – 7.44 (m, 2H, Ar), 7.33- 7.27 (m, 4H, Ar), 6.93-6.90 (m, 2 H, Ar), 5.88 (app. tt, 1H, H-3), 5.03 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9 Hz, H-2), 5.03-5.01 (m, 1H, H-1), 4.66 (ddd, 1H, $J_{3,4}$ = 9.4 Hz, $J_{4,5a}$ = 6.0 Hz, $J_{4,5b}$ 10.7 Hz, H-4), 4.04 (dd, 1H, $J_{5a,5b}$ = 11.2 Hz, H-5a), 3.89 (dd, 1H, H-5b), 3.39 (s, 3H, OCH_3), 2.15 (s, 3H, $ArCH_3$); ^{13}C NMR (100 MHz, CDCl3): δ 165.7, 164.9 (C=O), 133.4, 133.0, 132.8, 129.9, 129.7, 129.0, 128.8, 128.4, 128.1, 127.6 (C-Ar), 96.8 (C-1), 75.5 (C-4), 71.7 (C-2), 69.2 (C-3), 59.2 (C-5), 55.6 (OCH_3), 21.6 ($ArCH_3$).

Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-β-L-arabinopyranoside (16). Dibenzoate **15** (1.09 g, 2.07 mmol) was dissolved in anhydrous DMF (8 mL) and NaN_3 (5 eq., 10.4 mmol, 673 mg) was added. The reaction mixture was then stirred at 90 °C for 48 h under N_2. The resulting mixture was dissolved in $CHCl_3$ (100 mL) and washed with H_2O (3 × 100 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated. The residual oil was dissolved in EtOAc (3 mL) and *n*-hexane was added until a colourless solid precipitated. The crystals were collected and the filtrate was evaporated to dryness and to afford an additional product as a yellow solid (less pure product). The total yield of the product was 663 mg (81 %); R_f = 0.65 (toluene/ EtOAc, 5:1). β-L-arabinoside **16**: colourless needles, m.p. 102 - 104 °C (EtOAc – hexane; lit.[24] 104 – 105.5 °C); $[\alpha]_D^{20}$ +84.5 (c 0.49, $CHCl_3$); FTIR: 2112 (N_3), 1723 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 – 7.95 (m, 4H, Ph), 7.53 – 7.48 (m, 2H, Ph), 7.39 – 7.34 (m, 4H, Ph), 5.84 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 5.56 (dd, 1H, $J_{1,2}$ = 3.5 Hz, H-2), 5.12 (d, 1H, H-1), 4.26 – 4.24 (m, 1H, H-4), 4.05 (dd, 1H, $J_{4,5a}$ = 1.0 Hz, $J_{5a,5b}$ = 12.5 Hz, H-5a), 3.77 (dd, $J_{4,5b}$ = 2.0 Hz, 1H, H-5b), 3.41 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.8 (C=O), 133.5, 133.3, 129.9, 129.8, 129.2, 128.8, 128.5, 128.4 (Ph), 97.9 (C-1), 69.6 (C-3), 69.1 (C-2), 60.4 (C-4), 60.1 (C-5), 55.7 (OCH_3).

Methyl 4-azido-4-deoxy-β-L-arabinopyranoside (17). The azide **16** (663 mg, 1.67 mmol) was suspended in anhydrous

MeOH (12 mL), and a 1 M solution of NaOMe (3 mL) was added. The reaction mixture was then stirred at room temperature for 1 h (pH 10). The resulting mixture was neutralized by addition of strongly acidic ion exchange resin (Amberlite IR-120, H^+ form, pre-washed with anhydrous MeOH, 3 × 20 mL) in small portions to pH 7, and straight away filtered through a Büchner-funnel. The resin was washed with anhydrous MeOH (5 × 20 mL), and the combined filtrate and washings were concentrated in vacuo to give the crude product as a yellow solid. Flash chromatography ($CHCl_3$/ MeOH, 4:1 → 1:1 → 0:10) gave the diol **17** (280 mg, 88 %) as colourless needles, m.p. 86 – 88 °C ($CHCl_3$ – hexane; lit.[24] 86 – 88 °C; lit.[30] 81 – 83 °C); $[\alpha]_D^{20}$ +193 (c 0.53, $CHCl_3$); R_f = 0.68 ($CHCl_3$/ MeOH, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.77 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 3.95 – 3.90 (m, 2H, H-3, H-4), 3.81 (dd, 1H, $J_{4,5a}$ = 1.0 Hz, $J_{5a,5b}$ = 12.5 Hz, H-5a), 3.78 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.68 (dd, 1H, $J_{4,5b}$ = 2.5 Hz, H-5b), 3.41 (s, 3H, OCH_3), 2.47 (s, 1H, OH), 2.02 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 99.6 (C-1), 70.7 (C-3), 70.0 (C-2), 61.1 (C-4), 60.4 (C-5), 56.8 (OCH_3). LRMS: *m/z* = 212.0 $[M+Na]^+$; HRMS: *m/z* calcd for $C_6H_{11}N_3O_4Na$ $[M+Na]^+$: 212.0642; found: 212.0647.

Methyl 4-azido-4-deoxy-2,3-di-O-methyl-β-L-arabinopyranoside (18). Diol **17** (280 mg, 1.48 mmol) was dissolved in anhydrous DMF (15 mL), and NaH (8.4 eq., 0.30 g, 12.4 mmol, prewashed with hexanes) was added protionwise to the solution at room temperature. The resulting solution was stirred at room temperature under N_2 for 50 min. MeI (4.8 eq., 0.44 mL, 7.10 mmol) was then added dropwise and the mixture stirred for 2.5 h under N_2 at r.t. with protection from light. MeOH was added dropwise to decompose excess NaH. The resulting mixture was diluted with EtOAc (50 mL) and washed with H_2O (3 × 100 mL). The combined organic layers were washed with 1M HCl (100 mL), brine (100 mL), dried ($MgSO_4$) and concentrated to give the dimethyl ether **18** as a slightly yellow solid (300 mg, 93%); R_f = 0.84 ($CHCl_3$ / MeOH, 9:1); m.p. 230-234 °C (dec.; EtOAc – hexane); $[\alpha]_D^{20}$ + 5.6 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 3.99 – 3.97 (m, 1H, H-4), 3.76 (dd, 1H, $J_{4,5a}$ = 1.8 Hz, $J_{5a,5b}$ = 12.5 Hz, H-5a), 3.69 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.8 Hz, H-3), 3.63 (dd, 1H, $J_{4,5b}$ = 2.2 Hz, H-5b), 3.56 (dd, 1H, H-2), 3.51 (s, 3H, 2-OCH_3), 3.50 (s, 3H, 3-OCH_3), 3.40 (s, 3H, 1-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 98.4 (C-1), 78.8 (C-3), 77.8 (C-2), 60.2 (C-5), 59.3 (2-OCH_3), 59.0 (C-4), 58.2 (3-OCH_3), 55.6 (1-OCH_3); LRMS: *m/z* = 240.0 $[M+Na]^+$; HRMS: *m/z* calcd for $C_8H_{15}N_3O_4Na$ $[M+Na]^+$: 240.0955; found: 240.0944.

Methyl 4-deoxy-4-(2'-*tert*-butoxycarbonylamino)acetamido-2,3-di-*O*-methyl-β-L-arabinopyranoside (20). The dimethyl ether **18** (50 mg, 0.23 mmol) was dissolved in anhydrous MeOH (1 mL), and Pd/C (30 mg) was added. The mixture was stirred at room temperature under an atmosphere of H_2 for 19.5 h. The catalyst was then removed by filtration (celite), and the filtrate was concentrated to give the amine **19** (40 mg, 91 %) as light yellow solid; LRMS: *m/z* = 192.0 $[M+H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.81 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 3.80 (dd, 1H, $J_{4,5a}$ = 2.2 Hz, $J_{5a,5b}$ = 12.0 Hz, H-5a), 3.56 (dd, 1H, $J_{4,5b}$ = 2.3 Hz, H-5b), 3.48 (s, 3H, 2-OCH_3), 3.47 (m, 1H, H-2), 3.44 (s, 3H, 3-OCH_3), 3.44 (m, 1H, H-3), 3.40 (s, 3H, 1-OCH_3), 3.30 (m, 1H, H-4). A mixture of Boc-Gly-OH (40 mg, 0.21 mmol), HBTU (0.42 mmol, 159 mg) and DIPEA (0.21 mmol, 37 µL) was stirred at 0 °C for 5 minutes in anhydrous DMF (1 mL, dried over 3Å molecular sieves). The resulting solution was then added to a solution of amine **19** (40 mg, 0.21 mmol) and DIPEA (0.21 mmol, 37 µL) in anhydrous DMF (1 mL). The reaction mixture was stirred at 0 °C for 1 hour, and stirred at room temperature for 24 hours. The resulting mixture was then diluted with EtOAc (50 mL), and the mixture was washed with 1 M HCl (2 ×30 mL), saturated $NaHCO_3$ (2 × 30 mL) and saturated NaCl (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated to afford a yellow solid. The crude product was then purified by flash column chromatography ($CHCl_3$ / MeOH, 9:1) to give the carbamate **20** (15 mg, 19%) as colourless oil; R_f = 0.48 ($CHCl_3$ / MeOH, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.41 (d, 1H, *J* = 6.3 Hz, NHC=O), 5.12 (br s, 1H, $NHCO_2$), 4.79 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.40 (br s, 1H, H-4), 3.80 – 3.76 (m, 3H, CH_2, H-5a), 3.66 (dd, 1H, $J_{4,5b}$ = 2.1 Hz, $J_{5a,5b}$ = 12.2 Hz, H-5b), 3.62 (dd, $J_{3,4}$ = 4.6 Hz, $J_{2,3}$ = 9.4 Hz, 1H, H-3), 3.49 (s, 3H, 2-OCH_3), 3.41 (s, 3H, 1-OCH_3), 3.39 (s, 3H, 3-OCH_3), 3.26 (dd, 1H, H-2), 1.44 (s, 9H, Me_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8 (NHC=O), 156.1 ($NHCO_2$), 98.1 (C-1), 78.4 (Me_3C), 77.2 (C-2), 76.8 (C-3), 60.5 (C-5), 59.2 (2-OCH_3), 57.2 (3-OCH_3), 55.6 (1-OCH_3), 46.8 (C-4), 44.6 (CH_2), 28.3 (CH_3). LRMS: *m/z* = 349.1 $[M+H]^+$, 371.0 $[M+Na]^+$, 387.0 $[M+K]^+$; LRMS: *m/z* calcd for $C_{15}H_{28}N_2O_7Na$ $[M+Na]^+$: 371.1789; found: 371.1793.

Methyl 4-deoxy-4[3'-(fluoren-9-yl)methoxycarbonylamino]propanamido-2,3-di-*O*-methyl-β-L-arabinopyranoside (21). A mixture of Fmoc-β-Ala-OH (65 mg, 0.21 mmol), HBTU (0.42 mmol, 159 mg) and DIPEA (0.21 mmol, 37 µL) was stirred at 0 °C for 5 min in anhydrous DMF (1 mL, dried over 3Å molecular sieves). The resulting solution was then added to a solution of amine **19** (40 mg, 0.21 mmol) and DIPEA (0.21 mmol, 37 µL) in anhydrous DMF (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at room temperature for 22 h. The resulting mixture was then diluted with EtOAc (50 mL), and the mixture was washed with 1 M HCl (2 ×30 mL), saturated $NaHCO_3$ (2 × 30 mL) and saturated NaCl (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a yellow solid. The crude product was then purified by flash column chromatography ($CHCl_3$ / MeOH, 9:1) to give the carbamate **21** (35 mg, 32%) as colourless oil; R_f = 0.52 ($CHCl_3$ / MeOH, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74 (d, 2H, *J* = 7.6 Hz, ArH), 7.56 (d, 2H, *J* = 7.5 Hz, ArH), 7.37 (d, 2H, *J* = 7.4 Hz, ArH), 7.29 (d, 2H, *J* = 7.4 Hz, ArH), 5.93 (d, 1H, *J* = 7.4 Hz, NHC=O), 5.54 (br s, 1H, $NHCO_2$), 4.78 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1), 4.45 – 4.44 (m, 1H, H-4), 4.39 – 4.30 (m, 2H, $NHCO_2CH_2$), 4.18 (t, 1H, *J* = 6.95 Hz, CH-Fmoc), 3.79 (dd, 1H, $J_{4,5a}$ = 2.3 Hz, $J_{5a,5b}$ = 12.2 Hz, H-5a), 3.63 (dd, 1H, $J_{4,5b}$ = 2.5 Hz, H-5b), 3.61 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 4.7 Hz, H-3), 3.52 – 3.49 (m, 2H, CH_2NHCO_2), 3.45 (s, 3H, 2-OCH_3), 3.40 (s, 3H, 1-OCH_3), 3.39 (s, 3H, 3-OCH_3), 3.26 (dd, 1H, H-2), 2.46 (t, *J* = 5.5Hz, 2H, $NHCOCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (NHC=O), 156.5 ($NHCO_2$), 143.9, 141.3, 127.7, 127.0, 125.1, 120.0 (Ar), 98.1 (C-1), 77.2 (C-2), 76.9 (C-3), 66.8 ($NHCO_2CH_2$), 60.6 (C-5), 59.1 (2-OCH_3), 57.4 (3-OCH_3), 55.6 (1-OCH_3), 47.2 (CH-Fmoc), 46.8 (C-4), 37.3 (CH_2NHCO_2), 36.4 ($NHCOCH_2$). LRMS: *m/z* = 485.1 $[M+H]^+$, 507.1 $[M+Na]^+$, 523.1 $[M+K]^+$, 544.1 $[M+ACN+NH_4]^+$; HRMS: *m/z* calcd for $C_{26}H_{32}N_2O_7Na$ $[M+Na]^+$: 507.2101; found: 507.2105.

Methyl 4-deoxy-4-(4'-*tert*-butoxycarbonylamino)butanamido-2,3-di-*O*-methyl-β-L-

arabinopyranoside (22). A mixture of (Boc-γ–amino)butyric acid (43 mg, 0.21 mmol), HBTU (0.42 mmol, 159 mg) and DIPEA (0.21 mmol, 37 µL) was stirred at 0 °C for 5 minutes in anhydrous DMF (1 mL, dried over 3Å molecular sieves). The resulting solution was then added to a solution of amine **19** (40 mg, 0.21 mmol) and DIPEA (0.21 mmol, 37 µL) in anhydrous DMF (1 mL). The reaction mixture was stirred at 0 °C for 1 h, then at room temperature for 5 h. The resulting mixture was then diluted with EtOAc (50 mL), and the mixture was washed with 1 M HCl (2 ×30 mL), saturated $NaHCO_3$ (2 × 30 mL) and saturated NaCl (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a yellow solid. The crude product was then purified by flash chromatography ($CHCl_3$ / MeOH, 9:1) to give the carbamate **22** (11 mg, 13%) as colourless oil; R_f = 0.52 ($CHCl_3$ / MeOH, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.69 (br s, 1H, NHC=O), 4.82 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.74 (br s, 1H, $NHCO_2$), 4.48 – 4.45 (m, 1H, H-4), 3.78 (dd, 1H, $J_{4,5a}$ = 2.2 Hz, $J_{5a,5b}$ = 12.1 Hz, H-5a), 3.65 – 3.61 (m, 2H, H-5b, H-3), 3.50 (s, 3H, 2-OCH_3), 3.49 – 3.45 (m, 1H, H-2), 3.41 (s, 3H, 1-OCH_3), 3.38 (s, 3H, 3-OCH_3), 3.26 – 3.14 (m, 2H, CH_2NHCO$_2$), 2.26 (t, 2H, J = 7.0 Hz, NHCOCH_2), 1.79 (quintet, 2H, J = 6.9 Hz, CH_2CH$_2$NHCO$_2$), 1.43 (s, 9H, Me_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9 (NHC=O), 156.7 ($NHCO_2$), 98.1 (C-1), 79.4 (Me_3C), 77.2 (C-2), 76.9 (C-3), 61.0 (C-5), 59.0 (2–OCH_3), 57.0 (1–OCH_3), 55.5 (3–OCH_3), 46.7 (C-4), 39.3 (*C*H_2NHCO$_2$), 33.7 (NHCO*C*H$_2$), 28.4 (CH_3), 27.0 (*C*H_2CH$_2$NHCO$_2$). LRMS: *m/z* = 399.1 $[M+Na]^+$, 775.2 $[2M+Na]^+$; HRMS: *m/z* calcd for $C_{17}H_{32}N_2O_7Na$ $[M+Na]^+$: 399.2101; found: 399.2115.

Methyl 4-(2'-amino)acetamido-4-deoxy-2,3-di-*O*-methyl-β-L-arabinopyranoside (11). The carbamate **20** (15 mg, 0.043 mmol) was treated with cleavage solution (TFA/TES/dry DCM, 1:2:7, 0.5 mL) for 4 h at room temperature. The cleavage solution was then dried under N_2, and co-evaporated with a mixture of toluene/ *i*-PrOH (10:1) (5 × 5 mL) to afford the crude product. The crude amine was then purified using a reverse phase SPE C_{18} cartridge (5 g; 10 % ACN in H_2O, 0.1 % HCOOH → 100 % ACN, 0.1% HCOOH) to give the amine **11** as a colourless oil (10 mg, 93%). LCMS: t_R = 1.10 min, *m/z* = 271.39 $[M+Na]^+$; LRMS: *m/z* = 249.1 $[M+H]^+$, 271.0 $[M+Na]^+$; ^{1}H NMR (500 MHz, MeOD): δ 4.78 (1H, H-1, overlapped with MeOD solvent peak), 4.43-4.41 (m, 1H, H-4), 3.76 (dd, 1H, $J_{4,5a}$ = 2.0 Hz, $J_{5a,5b}$ = 12.1 Hz, H-5a), 3.65 (s, 2H, CH_2), 3.55 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.46 (dd, 1H, $J_{4,5b}$ = 2.7 Hz, H-5b), 3.40 (s, 3H, 3-OCH_3), 3.37 (dd, 1H, $J_{1,2}$ = 3.6 Hz, H-2), 3.32 (s, 6H, 3-OCH_3, 1-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4 (C=O), 99.5 (C-1), 78.5 (C-2), 78.4 (C-3), 62.0 (C-5), 59.1 (2- OCH_3), 57.8 (3-OCH_3), 55.8 (1-OCH_3), 48.4 (C-4), 41.5 (CH_2).

Methyl 4-(3'-amino)propanamido-4-deoxy-2,3-di-*O*-methyl-β-L-arabinopyranoside (12). The carbamate **21** (17.5 mg, 0.036 mmol) was dissolved in anhydrous DMF (2 mL), and PL-EDA resin (10 eq., 0.36 mmol, 72 mg) was added. The resulting mixture was stirred at 50 °C under N_2 for 10 minutes; it was then covered with aluminium foil and shaken for further 4 h at room temperature. Piperidine (50 µL) was added and the resulting mixture was shaken at 4 °C overnight (~ 17 h) in a cold room. The resin was then filtered off and washed with MeOH (5 × 20 mL) and dried. It was co-evaporated with toluene (5 × 20 mL) at room temperature to afford a white solid which was purified on a reverse phase SPE C_{18} cartridge (5g; 10% *i*-PrOH in H_2O, 0.1% HCOOH → 100% *i*-PrOH, 0.1% HCOOH) to give the amine **12** as a colourless oil (9 mg, 95%); LC/MS: t_R = 1.20 min, *m/z* = 285.33 $[M+Na]^+$; LRMS: *m/z* = 285.2 $[M+Na]^+$, 263.2 $[M+H]^+$. ^{1}H NMR (500 MHz, MeOD): δ 4.82 (1H, H-1, overlap with MeOD peak), 4.40 (br s, 1H, H-4), 3.74 (dd, 1H, $J_{4,5a}$ = 1.5 Hz, $J_{5a,5b}$ = 11.5 Hz, H-5a), 3.53 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.46 – 3.41 (m, 2H, H-5b, H-2), 3.40 (s, 3H, 2-OCH_3), 3.32 (s, 3H, 1-OCH_3), 3.32 (s, 3H, 3-OCH_3), 3.12-3.05 (m, 2H, NHCOCH_2), 2.62-2.56 (m, 2H, CH_2NH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4 (C=O), 98.1 (C-1), 77.1 (C-2), 77.0 (C- 3), 60.7 (C-5), 57.6 (2-OCH_3), 56.4 ((3-OCH_3), 54.4 (1-OCH_3), 46.7 (C-4), 35.7 (NHCO*C*H$_2$), 31.2 (CH_2NH_2).

Methyl 4-(4'-amino)butanamido-4-deoxy-2,3-di-*O*-methyl-β-L-arabinopyranoside (13). The carbamate **22** (12 mg, 0.032 mmol) was treated with cleavage solution (TFA/TES/dry DCM, 1:2:7, 0.5 mL) for 4 h at room temperature. The cleavage solution was then evaporated under N_2, and co-evaporated with a mixture of toluene/ *i*-PrOH (10:1) (5 × 5 mL) to afford the crude product. The crude amine was then purified on a reverse phase SPE C_{18} cartridge (5 g; 10% MeCN in H_2O, 0.1% HCOOH → 100% MeCN, 0.1% HCOOH) to give the amine **13** as a colourless oil (8 mg, 91%). LC/MS: t_R = 1.24 min, *m/z* = 299.76 $[M+Na]^+$; LRMS: *m/z* = 277.1 $[M+H]^+$; ^{1}H NMR (500 MHz, MeOD): δ 4.78 (1H, H-1, overlapped with MeOD solvent peak), 4.40 – 4.38 (m, 1H, H-4), 3.74 (dd, 1H, $J_{4,5a}$ = 2.5 Hz, $J_{5a,5b}$ = 12.0 Hz, H-5a), 3.51 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.44 – 3.41 (m, 2H, H-5b, H-2), 3.39 (s, 3H, 2-OCH_3), 3.31 (s, 3H, 3-OCH_3), 3.30 (s, 3H, 1-OCH_3), 2.92-2.88 (m, 2H, NHCOCH_2), 2.37 – 2.34 (m, 2H, CH_2NH$_2$), 1.87-1.80 (m, 2H, CH_2CH$_2$NH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3 (C=O), 98.1 (C-1), 77.0 (C-2), 76.9 (C-3), 60.7 (C-5), 57.6 (2-OCH_3), 56.3 (3-OCH_3), 54.4 (1-OCH_3), 38.9 (NHCO*C*H$_2$), 32.2 (CH_2NH_2), 23.1 (*C*H_2CH$_2$NH$_2$).

General experimental procedure for the stability of three model compounds under acidic conditions. A model compound (2mg) was dissolved in a mixture of 10 % TFA in H_2O (3.6 mL, pH 1). The resulting solution was stirred at 80 °C for 24 h. The reaction progress was analysed by TLC (*tert*-butanol/ acetic acid/ water, 2:1:1) and ^{1}H NMR and COSY spectroscopy. The compounds were too polar (t_R = 1.20 min, LCMS), and hence, the reaction progress could not be analysed by LC/MS. The resulting solution was concentrated under reduced pressure, and dried under high vacuum overnight. The reaction mixture was then examined by ^{1}H NMR and COSY spectroscopy.

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Electronic Supplementary Information (ESI) available: LC/MS data for compound **1**; ^{1}H and ^{13}C NMR data for compounds **1** and **2**; copies of ^{1}H

and ^{13}C NMR spectra for compounds **1-5** and **11-22**. See DOI: 10.1039/b000000x/

1. R. Hirschmann, L. Ducry and A. B. Smith, III, *J . Org. Chem.*, 2000, **65**, 8307-8316.
2. K. C. Nicolaou, J. M. Salvino, K. Raynor, S. Pietranico, T. Reisine, R. M. Freidinger and R. Hirschmann, in *Peptides, Chemistry, Structure and Biology: Proceedings of the 11th American Peptide Symposium.*, eds. J. E. Rivier and G. R. Marshall, ESCOM, Leiden, **1990**, pp. 881-884.
3. G. Abbenante, B. Becker, S. b. Blanc, C. Clark, G. Condie, G. Fraser, M. Grathwohl, J. Halliday, S. Henderson, A. Lam, L. Liu, M. Mann, C. Muldoon, A. Pearson, R. Premraj, T. Ramsdale, T. Rossetti, K. Schafer, G. L. Thanh, G. Tometzki, F. Vari, G. r. Verquin, J. Waanders, M. West, N. Wimmer, A. Yau, J. Zuegg and W. Meutermans, *J. Med. Chem.*, 2010, **53**, 5576-5586.
4. G. Thanh Le, G. Abbenante, B. Becker, M. Grathwohl, J. Halliday, G. Tometzki, J. Zuegg and W. Meutermans, *Drug Discov. Today*, 2003, **8**, 701-709.
5. R. Hirschmann, K. C. Nicolaou, S. Pietranico, E. M. Leahy, J. Salvino, B. Arison, M. A. Cichy, P. G. Spoors, W. C. Shakespeare, P. A. Sprengeler, P. Hamley, A. B. Smith, III, T. Reisine, K. Raynor, L. Maechler, C. Donaldson, W. Vale, R. M. Freidinger, M. R. Cascieri and C. D. Strader, *J. Am. Chem. Soc.*, 1993, **115**, 12550-12568.
6. R. Hirschmann, K. C. Nicolaou, S. Pietranico, J. Salvino, E. M. Leahy, P. A. Sprengeler, G. Furst, C. D. Strader, A. B. Smith, M. A. Cascieri, M. R. Candelore, C. Donaldson, W. Vale and L. Maechler, *J. Am. Chem. Soc.*, 1992, **114**, 9217-9218.
7. R. Hirschmann, W. Yao, M. A. Cascieri, C. D. Strader, L. Maechler, M. A. Cichy-Knight, J. Hynes, R. D. van Rijn, P. A. Sprengeler and A. B. Smith, *J. Med. Chem.*, 1996, **39**, 2441-2448.
8. W. Meutermans, G. Le Thanh and B. Becker, *ChemMedChem*, 2006, **1**, 1164-1194.
9. G. Le Thanh, G. Abbenante, G. Adamson, B. Becker, C. Clark, G. Condie, T. Falzun, M. Grathwohl, P. Gupta, M. Hanson, N. Huynh, P. Katavic, K. Kuipers, A. Lam, L. Liu, M. Mann, J. Mason, D. McKeveney, C. Muldoon, A. Pearson, P. Rajaratnam, S. Ryan, G. Tometzki, G. Verquin, J. Waanders, M. West, N. Wilcox, N. Wimmer, A. Yau, J. Zuegg and W. Meutermans, *J . Org. Chem.*, 2010, **75**, 197-203.
10. P. Rajaratnam, P. Gupta, P. Katavic, K. Kuipers, N. Huyh, S. Ryan, T. Falzun, G. B. Tometzki, L. Bornaghi, G. Le Thanh, G. Abbenante, L. Liu, W. Meutermans, N. Wimmer and M. L. West, *Aust. J. Chem.*, 2010, **63**, 693-699.
11. M. L. West, P. Andrews, T. E. Ramsdale, W. Meutermans, G. Le Thanh, C. Clark, G. Abbenante and L. Liu, Derivatives of monosaccharides for drug discovery. *PCT Int. Appl*. WO2004/014929 A1, 2004.
12. J. A. Davies, C. H. Hassall and I. H. Rogers, *J. Chem. Soc. C*, 1969, 1358-1363.
13. W. J. le Quesne and G. T. Young, *J. Chem. Soc.*, 1952, 594-597.
14. K. Poduska, G. S. Katrukha, A. B. Silaev and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1965, **30**, 2410- 2433.
15. A. M. Reverdito, M. Garcia, A. Salerno, M. Blanco and I. A. Perillo, *Spectrosc. Lett.*, 2001, **34**, 335-343.
16. P. C. de Visser, C. Govaerts, P. A. V. van Hooft, H. S. Overkleeft, A. Van Schepdael and J. Hoogmartens, *J. Chromatogr. A*, 2004, **1058**, 183-189.
17. T. R. Hurley, C. E. Colson, G. Hicks and M. J. Ryan, *J. Med. Chem.*, 1993, **36**, 1496-1498.
18. L. Arnoldi, A. Manzocchi, F. Magni, M. DelPuppo and M. G. Kienle, *Bioorg. Chem.*, 1997, **25**, 110-116.
19. J. Dheur, N. Ollivier and O. Melnyk, *Org. Lett.*, 2011, **13**, 1560-1563.
20. N. Ollivier, J. Dheur, R. Mhidia, A. Blanpain and O. Melnyk, *Org. Lett.*, 2010, **12**, 5238-5241.
21. W. Hou, X. Zhang, F. Li and C.-F. Liu, *Org. Lett.*, 2010, **13**, 386-389.
22. B. Müller, M. Blaukopf, A. Hofinger, A. Zamyatina, H. Brade and P. Kosma, *Synthesis*, 2010, 3143-3151.
23. Y. Tsuda, M. Nishimura, T. Kobayashi, Y. Sato and K. Kanemitsu, *Chem. Pharm. Bull.*, 1991, **39**, 2883 - 2887.
24. D. W. C. Jones, R. J. Nash, E. A. Bell and J. M. Williams, *Tetrahedron Lett.*, 1985, **26**, 3125-3126.
25. Y. Kondo, *Carbohydr. Res.*, 1982, **107**, 303-311.
26. P. J. Stevenson, *Org. Biomol. Chem.*, 2011, **9**, 2078-2084.
27. R. A. B. Bannard, A. A. Casselman, W. F. Cockburn and G. M. Brown, *Can. J. Chem.*, 1958, **36**, 1541-1549.
28. Y. Kondo, *Carbohydr. Res.*, 1982, **110**, 339-344.
29. J. G. Buchanan and R. Fletcher, *J. Chem. Soc. C*, 1966, 1926-1931.
30. A. J. Dick and J. K. N. Jones, *Can. J. Chem.*, 1965, **43**, 977-982.