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PAPER

Iminosugar–ferrocene conjugates as potential anticancer agents†

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We prepared a series of new iminosugar–ferrocene hybrids displaying potent inhibition of fucosidase (bovine kidney) and inactivation of MDA-MB-231 breast cancer cells proliferation at low micromolar concentrations. The synthetic route brought to light an unprecedented isomerisation of a 2-ethanalylpyrrolidine.

Introduction

Iminosugars are natural or synthetic carbohydrate mimics displaying potent affinities towards glycoenzymes.[1,2] Among the hundreds of known iminosugars, pyrrolidines like **1–3** (Fig. 1), which share a specific fucose-like substituent distribution, are highly potent nanomolar inhibitors of alpha-L-fucosidase (AFU).[3] This particular enzyme is associated with many disorders including inflammation or viral infection.[4] Moreover, increasing fucosylation contributes to several abnormal characteristics of tumor cells, regarding adhesion and growth, and AFU levels are significantly increased in certain malignant tissues.[4e,f] It is not firmly established whether the overexpression of AFU is a cause or a consequence of cancer development. However, it seemed rational to explore the possibility of using the fucose-binding protein AFU as a target for the selective delivery of a cytotoxic molecule towards cancer cells. Ferrocene (Fc) is a remarkable pharmacophore, exhibiting physicochemical properties that accommodate biological uses.[5] Whereas Fc has no biological effect by itself, some ferrocene conjugates were shown to display antitumor, antimalarial or antifungal properties.[6]

Combining our efforts towards innovative fucosidase inhibitors and ferrocene conjugates, we designed Fc–iminosugar hybrids **4a–c** (Fig. 1) as a new class of potential anticancer agents. Their structures result from the association of a cytotoxic Fc moiety with a *fuco*-configured polyhydroxy-pyrrolidine playing the role of the drug-carrier targeting AFU.

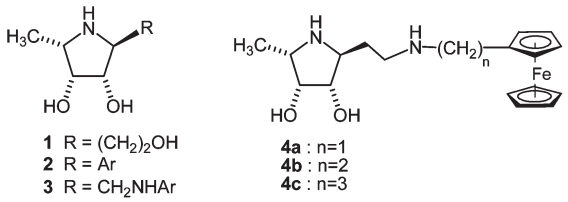

Fig. 1 Structures of iminosugars **1–4**.

Results and discussion

The anticipated strategy for the synthesis of organometallic **4** relied on a reductive amination between a ferrocenylamine and a protected polyhydroxy-pyrrolidine featuring an ethanalyl side chain. We wished to prepare first a series of target compounds, varying the length of the flexible linker connecting Fc to the iminosugar in order to establish structure–activity relationships. To this aim, we envisioned introducing ferrocenylamines **5a–c** of general structure $Fc(CH_2)_nNH_2$ [**5a**: $n = 1$; **5b**: $n = 2$; **5c**: $n = 3$] into our synthetic plan. Satisfactorily, these compounds were easily prepared following reported procedures.[7] The required aldehydo-pyrrolidine, in turn, could be obtained from unsaturated precursors, as exemplified by former examples from the literature.[8] Thus, we focused our first efforts on the preparation of C-2 allyl-pyrrolidine **8a** (Scheme 1), featuring a (2*S*) configuration at the pseudo-anomeric position. Indeed, this definite configuration would provide adequate orientation of the C-2 substituent to induce strong binding to AFU, the (2*R*) isomers being usually much less active as fucosidase inhibitors.[3c]

The synthesis of **8a** started with glycosylamine **6**, prepared in a mere 5 steps from D-ribose according to a reported route.[3b] Allyl-magnesium chloride reacted efficiently with **6**, affording ring-opening products **7a** and **7b** in pure form after separation

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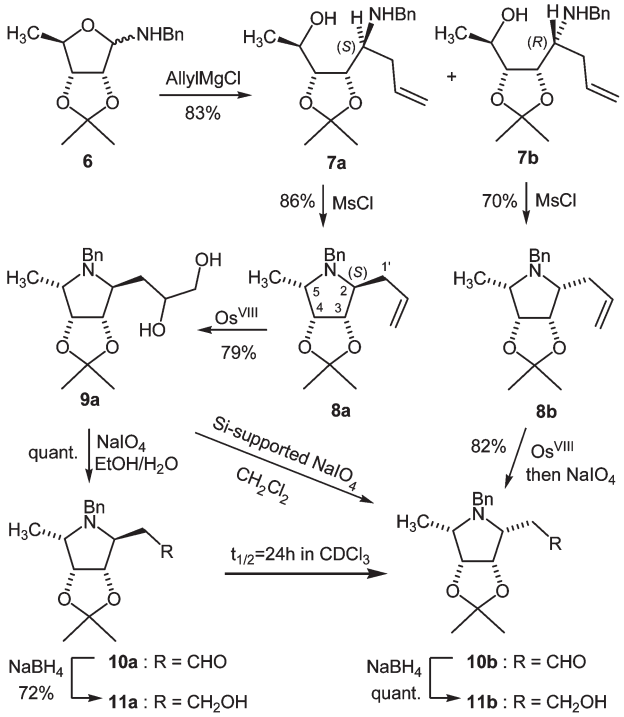
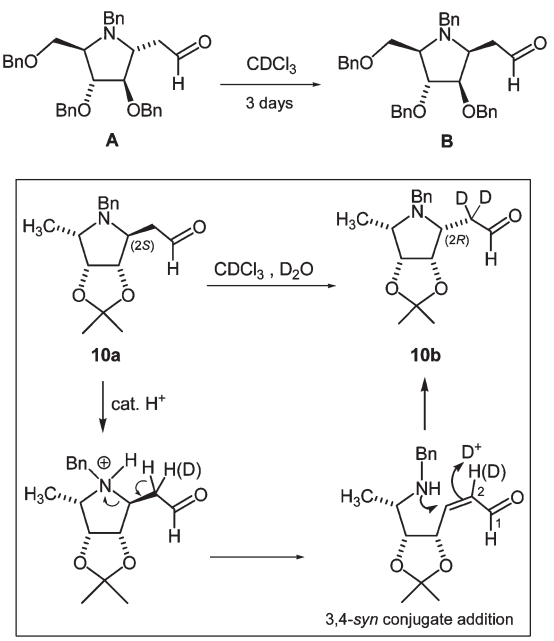


Scheme 1 Synthesis of ethanalylpyrrolidines.

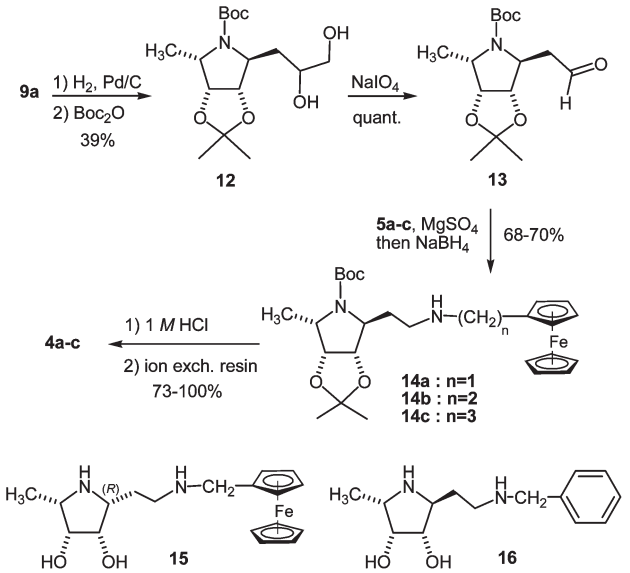
by silica gel chromatography (83% yield). However, the addition occurred with modest facial stereoselectivity (60% de). Determination of the configuration at the newly created stereocenter in **7a** and **7b** was performed after subsequent transformation into the corresponding pyrrolidines **8a** and **8b** respectively (a process effected with MsCl and resulting in inversion at C-5) and proved the major isomer to have the expected (2*S*) configuration. Hence, the nucleophilic attack at the anomeric carbon of **6** is *anti*-selective, a result that is in agreement with previous observations on the addition of organometallics to isopropylidene-protected ribosyl-amines.[3b,9] Unfortunately, we failed to increase the stereoselectivity by using a Barbier-type reaction with indium and allyl bromide as the allylating agent.[10] Under these conditions, the reaction did not afford the expected aminoalcohol **7** but mainly unwanted degradation products.

Next, the synthesis of the required aldehyde from allyl-pyrrolidine **8a** was experienced, following a standard osmylation/oxidation procedure.[11] When subjected to the Upjohn conditions (cat. OsO_4, *N*-methyl morpholine *N*-oxide as co-oxidant), allyl-pyrrolidine **8a** afforded diol **9a** (dr, 1 : 1) in only 32% yield. The use of commercial alpha AD-mix as an osmium^{VIII} source led to significantly better results, the reaction affording **9a** (dr, 6 : 4) in 79% isolated yield. The oxidative cleavage of diol **9a** was then studied (Scheme 1). The use of silica-supported sodium periodate has proven successful for such a transformation, and has the advantage of being carried out in CH_2Cl_2.[11] However, when a solution of **9a** was stirred in the presence of a suspension of silica supported $NaIO_4$, the diol reacted within 1 h to give a 1 : 1 mixture of *two* aldehydes, as deduced from the ^{1}H-NMR spectrum of the crude reaction mixture. Surprisingly, after purification on silica gel only *one* aldehyde remained, which was isolated in 82% yield. To confirm the stereochemistry, a $NaBH_4$ reduction was effected yielding alcohol **11b** as the sole product, which features the (2*R*) configuration. Obviously, the formation of intermediate aldehyde **10b** resulted from an unexpected isomerisation of the pre-formed (2*S*) isomer **10a** during the reaction or purification. These observations suggest a critical role of silica in this intriguing transformation. We thus performed the periodate cleavage under more standard conditions, using EtOH–water as the solvent and powdered sodium periodate as the reagent. Gratifyingly, only aldehyde **10a** was formed and isolated almost pure after extraction with CH_2Cl_2. The structure of **10a** was correlated with known alcohol **11a** after reduction with sodium borohydride, which ascertained the (2*S*) configuration.[12] However, aldehyde **10a** was also prone to isomerisation in a $CDCl_3$ solution ($t_{1/2}$ = 24 h at room temperature), though much more slowly than in the presence of silica. At the same time, when (2*R*) configurated allyl-pyrrolidine **8b** was subjected to the same sequence of reactions (osmylation–periodate cleavage) the expected aldehyde **10b** was obtained quantitatively as the sole product. This unusual transformation of **10a** into **10b** has no precedent in the literature and further experiments were conducted to determine a possible mechanism for this transformation (Scheme 2). Firstly, we prepared the all-*trans* aldehyde **A** by treating the known diol precursor[13] with sodium periodate under the conditions stated above. As expected, aldehyde **A** epimerised to **B** quantitatively after 3 days at room temperature in a $CDCl_3$ solution. No isomerization occurred with **B** under the same conditions. This transformation seems thus general and could be applicable to other ethanalyl-pyrrolidines. Furthermore, when **10a** was subjected to epimerisation in the presence of deuterated water, proton exchange occurred strictly at the *exo*-cyclic enolizable position and not at C-2, affording deuterated **10b** (Scheme 2).

As a consequence, the transformation of **10a** into **10b** implies the breaking of the C–N bond, which could reasonably occur *via* elimination. This step could be catalysed by traces of acid



Scheme 2 Proposed mechanism accounting for epimerization of 2-ethanalylpyrrolidines.



Scheme 3 Coupling and deprotection.

present either on silica or in $CDCl_3$. A subsequent aza-Michael conjugate addition could account for the re-formation of the pyrrolidine ring. Interestingly, such additions were previously shown to be 3,4-*syn* selective,[14] which is in agreement with the results obtained either with **A** or with **10a**.

Nevertheless, the assistance of the neighboring amino group might act as a driving force, thus we turned to a *t*-butylcarbamate protecting group at nitrogen, to prevent this undesired epimerisation. Hydrogenolysis of diol **9a** followed by reaction with di-*tert*-butyl dicarbonate afforded diol **12** in 39% yield for the two steps (Scheme 3).

Aldehyde **13** was the only product obtained by oxidative cleavage of **12**, whatever the source of $NaIO_4$ used, and proved stable either on silica gel or in solution. The stereochemistry of **13** was unequivocally confirmed by chemical correlation after its transformation ($NaBH_4$, then 1 M HCl) to the known iminosugar **1**.[12] Reductive amination was performed next with aldehyde **13** and amines **5a–c** respectively, by stirring both reagents in CH_2Cl_2 in the presence of $MgSO_4$ as a dehydrating agent. After filtration and evaporation, MeOH was added and the resulting imine was thoroughly reduced with sodium borohydride to afford ferrocenyliminosugars **14a–c** in 68–70% yield after purification. Final deprotection was achieved by stirring overnight with aqueous HCl, and target compounds **4a–c** were obtained in a pure form after neutralization and chromatography over silica gel. A similar sequence of reactions afforded compound **15** (structure shown in Scheme 3), the C-2 epimer of **4a** that features the "wrong" (2*R*) configuration. Finally, we prepared also compound **16**, a non-Fc analogue of **4a**, by using benzylamine in the reductive amination with aldehyde **13**. Iminosugar **16** could play the role of a control molecule to evaluate the influence of Fc on either antifucosidase or antiproliferative activity.

Preliminary enzymatic and anticancer evaluations were performed on Fc–iminosugars **4a–c**, **15**, **16**. All the compounds displayed micromolar inhibition towards fucosidase from bovine kidney (Table 1), **4c** being more active (IC_{50} = 1.2 μM) than **4a** (IC_{50} = 1.6 μM) or **4b** (IC_{50} = 1.6 μM). The presence of the

Table 1 Antifucosidase activity of compounds **4a–c**, **15**, **16**[a]

Entry	Compound	IC_{50} [b] (μM)
1	**4a**	1.6 ± 0.1
2	**4b**	1.6 ± 0.2
3	**4c**	1.2 ± 0.1
4	**15**	43.8 ± 4.2
5	**16**	4.5 ± 0.5

[a] Inhibition of fucosidase (bovine kidney) at 2 mM substrate concentration. [b] Determined according to Dixon plots by assaying five concentrations of each inhibitor.

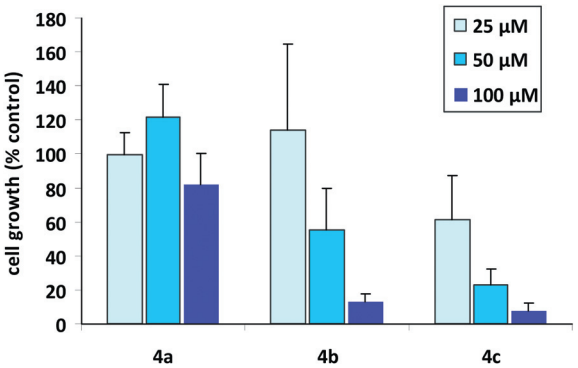


Fig. 2 Effect of ferrocene–iminosugar conjugates **4a–c** on MDA-MB-231 breast cancer cells proliferation.

bulky Fc functionality did not impede binding of the pyrrolidine ligand to fucosidase. The Fc containing **4a** was even a better inhibitor than its phenyl counterpart **16** (IC_{50} = 4.5 μM). As anticipated, the (2*R*) configuration led to a less potent inhibitor: compound **15** (IC_{50} = 43.8 μM) was at least 10-fold less active than the other iminosugars towards fucosidase. The anti-proliferative effect was next analyzed using the hormone-independent breast cancer cell line MDA-MB-231, previously shown to be sensitive to ferrocene derivatives.[4g,15] Cell growth data are reported in Fig. 2 and are expressed as percentage of untreated control. To our delight, compounds **4a–c** had a significant effect on MDA-MB-231 breast cancer cells proliferation in a concentration-dependent manner (Fig. 2). Here again, the best results were obtained with Fc–iminosugar **4c**, which displayed 77% growth inhibition at 50 μM and led to almost complete inactivation at 100 μM. Interestingly, **4c** was up to 10-fold more potent as a cytotoxic agent than the reference drug cisplatin (50% growth inhibition at 226 μM) towards the studied cell lines.[16] In return, compounds **15** and **16** showed no antiproliferative activity up to the maximum tested concentration of 100 μM (data not reported in Fig. 2).

Conclusions

The synthesis of iminosugar–ferrocene conjugates was achieved by reductive amination of an *N*-Boc protected ethanalylpyrrolidine with ferrocenylamines. The carbamate protection proved crucial to prevent epimerisation at C-2 of the pyrrolidine ring. Such a spontaneous isomerisation was observed with the *N*-Bn

protected analogues, and could occur *via* a elimination/aza Michael sequence. The iminosugar–ferrocene hybrids displayed potent inhibition towards fucosidase and showed a significant effect on the growth of MDA-MB-231 breast cancer cells proliferation. These first results demonstrate the potential of ferrocene–iminosugars as anticancer agents. Work is under progress to prepare a new set of Fc–iminosugars and non-Fc analogues to elucidate the exact mechanism of action of this novel family of anticancer agents and to study the possibility of targeting selectively cancer cells through recognition by fucosidase.

Experimental section

General considerations

All reactions were performed under argon. The reagents and solvents were commercially available in high purity and used as received. Silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid, *p*-anisaldehyde or an aqueous solution of $KMnO_4$ (2%)–Na_2CO_3 (4%), followed by heating. Flash column chromatography was performed over silica gel M 9385 (40–63 μm) Kieselgel 60. NMR spectra were recorded on Bruker AC 250 (250 MHz for ^1H, 62.5 MHz for ^{13}C) or 600 (600 MHz for ^1H, 150 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded with an IR^{TM} plus MIDAC spectrophotometer and are expressed in cm^{-1}. Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241 polarimeter in the specified solvents. High resolution mass spectra (HRMS) were performed on a Q-TOF Micro micromass positive ESI (CV = 30 V).

General procedure for the synthesis of allylpyrrolidines 8a, 8b

Allylmagnesium chloride (15 ml of a commercial 2 M solution in THF, 30 mmol) was added to a stirred solution of glycosylamine **6** (2.059 g, 7.88 mmol) in THF (20 ml) at 0 °C, and the resulting mixture was left to react at rt for 7 h. Saturated NH_4Cl was then added and the solution was extracted with Et_2O (3 × 20 ml). The combined organic layers were dried ($MgSO_4$), evaporated and aminoalcohols **7a**, **7b** were separated and purified by FC (Et_2O–petroleum ether, 5 : 5) to yield **7a** (R_f = 0.46, 1.545 g, 64%) and **7b** (R_f = 0.26, 0.467 g, 19%) as yellow oils. To a solution of pure amino alcohol (1.068 g, 3.50 mmol) in pyridine (4.5 ml) and THF (4.5 ml) at 0 °C, MsCl (664 μl, 8.72 mmol, 2.45 equiv.) was added dropwise. The mixture was stirred for 2 h at 0 °C and for an additional 2 h at room temperature. Then a saturated solution of NH_4Cl and Et_2O were successively added at 0 °C and the resulting organic phase was separated. The aqueous layer was extracted with Et_2O and the combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography (Et_2O–petroleum ether: 2 : 8) to yield pure allylpyrrolidine.

8a (86%, yellow oil): R_f = 0.56 (Et_2O–petroleum ether: 2 : 8); $[\alpha]_D^{20}$ = 63.2 (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 700, 1064, 1161, 1210, 1378, 2933, 2984; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.12 (d, 3H, $^3J_{HH}$ = 6.0 Hz, 6-H), 1.33 (s, 3H, iPr), 1.56 (s, 3H, iPr), 1.76–1.97 (m, 1H, 1′-H), 2.22–2.42 (m, 1H, 1′-H), 2.92 (quint, 1H, $^3J_{HH}$ = 6.0, 5-H), 3.08 (dd, $^3J_{HH}$ = 10.5, 3.2 Hz, 2-H), 3.55 (d, 1H, $^2J_{HH}$ = 14.0 Hz, CH_2-Ph), 3.91 (d, 1H, $^2J_{HH}$ = 14.0 Hz, CH_2-Ph), 4.43 (d, 1H, $^3J_{HH}$ = 6.5 Hz, 3-H), 4.52 (t, 1H, $^3J_{HH}$ = 6.5 Hz, 4-H), 4.64–5.10 (m, 2H, 3′-H), 5.53–5.74 (m, 1H, 2′-H), 7.16–7.45 (m, 5H, Ar); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 12.6 (6-C), 25.5, 26.4 (iPr), 28.9 (1′-C), 50.2 (CH_2-Ph), 58.6 (5-C), 63.4 (2-C), 81.6, 82.0 (3,4-C), 111.4 (iPr-C), 117.1 (3′-C), 126.6 and 128.1 (Ar-C), 135.2 (2′-C), 138.6 (Ar-C); HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{26}NO_2$ 288.1964; found 288.1968.

8b (70%, yellow oil): R_f = 0.53 (Et_2O–petroleum ether: 2 : 8); $[\alpha]_D^{20}$ = −58.2 (*c* 1, $CHCl_3$); IR (film) ν_{max} 702, 997, 1124, 1208, 1239, 1369, 1378, 2933, 2985; ^1H NMR ($CDCl_3$, 250 MHz) δ 1.15 (d, 3H, $^3J_{HH}$ = 6.0 Hz, 6-H), 1.31 (s, 3H, iPr), 1.53 (s, 3H, iPr), 2.17–2.50 (m, 4H, 2,5,1′-H), 3.75 (s, 2H, CH_2-Ph), 4.40 (t, 1H, $^3J_{HH}$ = 5.5 Hz, 4-H), 4.49 (t, 1H, $^3J_{HH}$ = 5.5 Hz, 3-H), 5.00–5.07 (m, 1H, 3′-Hb), 5.10–5.17 (m, 1H, 3′-Ha), 5.75–6.02 (m, 1H, 2′-H), 7.15–7.38 (m, 5H, Ar); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 13.4 (6-C), 26.1 (iPr), 26.6 (iPr), 32.4 (1′-C), 53.3 (CH_2-Ph), 62.2 (5-C), 67.3 (2-C), 79.5 (3-C), 81.1 (4-C), 111.2 (iPr-C), 116.9 (3′-C), 127.2, 128.5 and 129.3 (Ar-C), 136.0 (2′-C), 138.6 (Ar-C); HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{26}NO_2$ 288.1964; found 288.1956.

Synthesis of diol 9a. A suspension of alpha AD-mix (3.3 g) in acetone–distilled water (50 : 50, 20 ml) was added to allylpyrrolidine **8a** (450 mg, 1.568 mmol) at 0 °C. The resulting mixture was left to react at 4 °C for 35 h. Na_2SO_3 (2 g) was then added and the mixture was stirred for an additional 45 min. The solution was extracted with EtOAc (3 × 20 ml). The combined organic layers were dried ($MgSO_4$), filtered, evaporated and the residue was purified by silica gel chromatography (EtOAc) to yield diol **9a** (395 mg, 79%, yellow oil) as a mixture of two diastereomers (79%, orange oil): R_f = 0.24 (EtOAc); IR (film) ν_{max} 734, 1057, 1377, 2933, 2982; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{27}NO_4$ 322.2018; found 322.2034; major isomer: ^1H NMR ($CDCl_3$, 250 MHz) δ 1.20 (d, 1H, *J* = 6.8 Hz, 6-H), 1.32 (s, 3H, iPr), 1.36–1.54 (m, 1H, 1′-Ha), 1.56 (s, 3H, iPr), 1.64–1.72 (m, 1H, 1′-Hb), 2.96–3.12 (m, 1H, 3′-Ha), 3.20–3.59 (m, 3H, 2,5-H and 3′-Hb), 3.85 (dd, 2H, *J* = 78.7, 10.1 Hz, CH_2-Ph), 4.51–4.70 (m, 2H, 3,4-H), 7.23–7.40 (m, 5H, Ar); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 10.71 (6-C), 24.55 (iPr), 26.28 (iPr), 29.14 (1′-C), 51.86 (CH_2-Ph), 57.95 (5-C), 63.20 (2-C), 66.07 (3′-C), 70.71 (2′-C), 81.86 (4-C), 84.49 (3-C), 112.26 (iPr-C), 128.58, 128.61 and 129.34 (3 × Ar), 138.77 (Ar-C); minor isomer: ^1H NMR ($CDCl_3$, 250 MHz) δ 1.21–1.28 (m, 1H, 1′-Ha), 1.38 (d, 1H, *J* = 6.9 Hz, 6-H), 1.32 (s, 3H, iPr), 1.36–1.54 (m, 1H, 1′-Hb), 1.62 (s, 3H, iPr), 3.23–3.29 (m, 2H, 2′-H), 3.43–3.52 (m, 3H, 2,3′-H), 3.85 (dd, 2H, *J* = 78.7, 10.1 Hz, CH_2-Ph), 4.35–4.37 (m, 1H, 3-H), 4.59–4.63 (m, 1H, 4-H), 7.23–7.40 (m, 5H, Ar). ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 11.09 (6-C), 23.53 (iPr), 26.49 (iPr), 30.91 (1′-C), 53.22 (CH_2-Ph), 58.30 (5-C), 65.31 (2-C), 66.70 (3′-C), 71.23 (2′-C), 83.55 (4-C), 86.25 (3-C), 111.63 (iPr-Cq), 129.07, 129.34, 129.90 (3 × Ar), 139.08 (Ar-Cq).

Synthesis of aldehyde 13. A mixture of diol **9a** (392 mg, 1.22 mmol), HCO_2NH_4 (538 mg, 8.54 mmol, 7 equiv.) and

Pd/C 10% (392 mg) in MeOH (12 ml) was stirred at 60 °C for 1.5 h. Filtration over celite and purification by silica gel chromatography (DCM–MeOH: 9 : 1) yielded the *N*-debenzylated diol (158 mg, 56%, colorless oil), which was added to a solution of Et_3N (243 µl, 2.392 mmol, 3.5 equiv.) and Boc_2O (164 mg, 0.766 mmol, 1.1 equiv.) in CH_2Cl_2 (7 ml) at 0 °C. The mixture was allowed to warm slowly to room temperature and was stirred for 24 h. Distilled water (20 ml) was then added and the aqueous layer was extracted with DCM (3 × 20 ml). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc) to afford **12** (158 mg, 70%, colourless oil). To this diol **12** (75 mg, 0.226 mmol) in 4.5 ml of a 2 : 1 EtOH–water solution was added $NaIO_4$ (141 mg, 0.661 mmol, 2.9 equiv.). The solution was stirred at rt for 1 h, then water (10 ml) and DCM were added. After separation of the layers, the aqueous phase was washed with DCM (3 × 10 ml). The combined organic layers were dried ($MgSO_4$), filtered and evaporated to afford the crude aldehyde **13** (67 mg, 100%, yellow oil) which was used in the next step without further purification.

General procedure for the synthesis of iminosugar–ferrocene conjugates 14a–c, 4a–c, 15, 16

To a solution of aldehyde **13** (30 mg, 0.100 mmol) in DCM (2 ml), $MgSO_4$ (119 mg, 1.00 mmol, 10 equiv.) and the ferrocenylamine **5** (1.2 equiv.) were successively added. The solution was stirred at rt for 5 h. After filtration and concentration, the resulting material was dissolved in MeOH (2 ml) and $NaBH_4$ (5 mg, 0.130 mmol, 1.3 equiv.) was added at 0 °C. This solution was stirred and left to warm to rt overnight. Saturated NH_4Cl and EtOAc were successively added at 0 °C and the resulting organic layer was separated. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography (EtOAc–MeOH: 19 : 1 → 8 : 2) to yield pure ferrocenyliminosugar **14**.

14a (68%, yellow oil): R_f = 0.25 (EtOAc–MeOH: 19 : 1); $[\alpha]_D^{20}$ = +31.9 (*c* 0.84, $CHCl_3$); IR (film) ν_{max} 1027, 1107, 1144, 1171, 1244, 1373, 1689, 2934, 2981; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{38}FeN_2O_4$ 499.2259; found 499.2250.

14b (70%, yellow oil): R_f = 0.43 (EtOAc–MeOH: 19 : 1); $[\alpha]_D^{20}$ = +28.9 (*c* 1, $CHCl_3$); IR (film) ν_{max} 1026, 1107, 1143, 1171, 1245, 1373, 1690, 2932, 2979; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{27}H_{40}FeN_2O_4$ 513.2416; found 513.2410.

14c (70%, yellow oil): R_f = 0.23 (EtOAc–MeOH: 8 : 2); $[\alpha]_D^{20}$ = +24.4 (*c* 1, $CHCl_3$); IR (film) ν_{max} 754, 1025, 1107, 1373, 1688, 2932; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{28}H_{42}FeN_2O_4$ 527.2572; found 527.2575.

The carbamate protecting group at nitrogen induced very large NMR signals (see copies of NMR spectra in the ESI†). Consequently, compounds **14a–c** were fully characterized in their deprotected form after treatment of a solution of ferrocenyliminosugar **14** (0.0684 mmol) in MeOH (1 ml) with 1 M HCl (1 ml). The mixture was stirred at 40 °C overnight. After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and evaporated. Purification by silica gel column chromatography (DCM–MeOH: 8 : 2 → $CHCl_3$–MeOH–NH_4OH: 6 : 4 : 1) yielded ferrocenyliminosugar **4** as a yellow film.

4a (98%): $[\alpha]_D^{20}$ = −17.4 (*c* 1, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ 1.25 (d, 3H, *J* = 6.8 Hz, 6-H), 1.93–2.13 (m, 2H, 1′-H), 3.11 (t, 2H, *J* = 7.7 Hz, 2′-H), 3.31 (td, 1H, *J* = 2 × 7.9, 6.8 Hz, 2-H), 3.50–3.55 (m, 1H, 5-H), 4.02–4.09 (m, 2H, 3-H, 4-H), 4.11 (s, 2H, NCH_2Fc), 4.26–4.46 (m, 9H, Fc); ^{13}C NMR (125 MHz; CD_3OD) δ 13.8 (6-C), 31.1 (1′-C), 46.8 (2′-C), 48.9 (NCH_2Fc), 56.6 (5-C), 61.5 (2-C), 69.8, 69.8, 70.5, 70.6 (Fc), 74.4 (4-C), 79.0 (3-C), 82.3 (Cq-Fc); HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{27}FeN_2O_2$ 359.1422; found 359.1419.

4b (73%): $[\alpha]_D^{20}$ = −7.2 (*c* 0.5, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ 1.33 (d, 1H, *J* = 6.8 Hz, 6-H), 2.12–2.22 (m, 2H, 1′-H), 2.80 (t, 2H, *J* = 7.2 Hz, NCH_2CH_2Fc), 3.16–3.28 (m, 4H, 2′-H and NCH_2CH_2Fc), 3.43 (q, 1H, *J* = 7.5 Hz, 2-H), 3.68–3.77 (m, 1H, 5-H), 4.12 (t, 1H, *J* = 3.2 Hz, 4-H), 4.17 (dd, 1H, *J* = 7.5, 3.2 Hz, 3-H), 4.21–4.33 (m, 9H, Fc); ^{13}C NMR (125 MHz; CD_3OD) δ 12.7 (6-C), 28.0 (NCH_2CH_2Fc), 29.1 (1′-C), 46.3, 50.0 (2′-C, NCH_2CH_2Fc), 57.9 (5-C), 60.1 (2-C), 68.9, 69.3, 69.7 (Fc), 73.4 (4-C), 78.0 (3-C), 84.5 (Cq-Fc); HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{19}H_{29}FeN_2O_2$ 373.1578; found 373.1578.

4c (97%): $[\alpha]_D^{20}$ = −12.9 (*c* 0.86, MeOH); ^{1}H NMR (D_2O, 600 MHz) δ 1.34 (d, 3H, *J* = 6.7 Hz, 6-H), 1.88–1.95 (m, 2H, $NCH_2CH_2CH_2Fc$), 2.18–2.21 (m, 2H, 1′-H), 2.47 (t, 2H, *J* = 7.5 Hz, $NCH_2CH_2CH_2Fc$), 3.05–3.13 (m, 2H, $NCH_2CH_2CH_2Fc$), 3.14–3.28 (m, 2H, 2′-H), 3.47 (q, 1H, *J* = 7.5 Hz, 2-H), 3.72–3.77 (m, 1H, 5-H), 4.12–4.14 (m, 1H, 4-H), 4.16–4.18 (m, 1H, 3-H), 4.18–4.26 (m, 9H, Fc); ^{13}C NMR (126 MHz; CD_3OD) δ 12.6 (6-C), 27.7, 29.2, 29.3 (1′-C, $NCH_2CH_2CH_2Fc$), 46.1, 49.9 (2′-C, $NCH_2CH_2CH_2Fc$), 58.1 (5-C), 59.8 (2-C), 68.5, 69.1, 69.6 (3 × Fc), 73.5 (4-C), 78.1 (3-C), 88.3 (Cq-Fc); HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{31}FeN_2O_2$ 387.1735; found 387.1752.

15 (100%): $[\alpha]_D^{20}$ = 2.4 (*c* 0.33, H_2O); ^{1}H NMR (D_2O, 600 MHz) δ 1.15 (d, 3H, *J* = 6.8 Hz, 6-H), 1.76 (td, 1H, *J* = 15.3, 7.8 Hz, 1′-Ha), 1.94 (dt, 1H, *J* = 14.0, 7.8 Hz, 1′-Hb), 2.96 (t, 2H, *J* = 7.8 Hz, 2′-H), 3.11 (q, 1H, *J* = 6.4 Hz, 2-H), 3.19 (quint, 1H, *J* = 6.4 Hz, 5-H), 3.94 (s, 2H, NCH_2Fc), 4.13 (t, 1H, *J* = 5.2 Hz, 3-H), 4.22 (t, 1H, *J* = 5.2 Hz, 4-H), 4.25–4.44 (m, 9H, Fc); ^{13}C NMR (126 MHz, MeOD) δ 14.4 (6-C), 27.1 (1′-C), 46.8 (2′-C), 49.0 (3′-C), 56.7 (5-C), 59.6 (2-C), 69.8, 70.0, 70.7 (3 × Fc), 73.6 (3-C), 73.8 (4-C); ESI-HRMS: calcd for $C_{18}H_{27}FeN_2O_2$ ($[M + H]^+$) 359.1422; found 359.1430.

16 (100%): $[\alpha]_D^{20}$ = −31.6 (*c* 0.5, MeOH); ^{1}H NMR (D_2O, 600 MHz) δ 1.19 (d, 1H, *J* = 6.8 Hz, 6-H), 1.82–1.94 (m, 1H, 1′-Ha), 1.94–2.07 (m, 1H, 1′-Hb), 2.91–3.06 (m, 2H, 2′-H), 3.24 (dd, 1H, *J* = 6.1, 7.8 Hz, 2-H), 3.36–3.44 (m, 1H, 5-H), 3.95–4.02 (m, 2H, 3,4-H), 4.02–4.08 (m, 2H, 3′-H), 7.40–7.49 (m, 5H, Ar); ^{13}C NMR (63 MHz, MeOD) δ 12.99 (6-C), 30.34 (1′-C), 45.49 (2′-C), 51.99 (3′-C), 55.66 (5-C), 59.09 (2-C), 73.51 (4-C), 77.53 (3-C), 129.23, 129.53, 129.73 (Ar), 134.58 (Ar-Cq); ESI-HRMS: calcd for $C_{18}H_{27}FeN_2O_2$ ($[M + H]^+$) 251.1760; found 251.1768.

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