Total Synthesis of (+)-Kifunensine, a Potent Glycosidase Inhibitor

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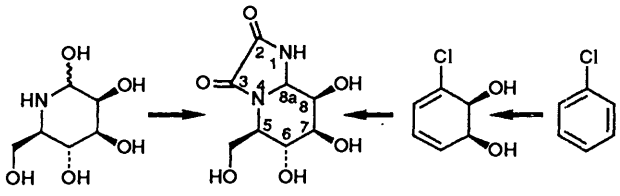
(+)-Kifunensine, a potent inhibitor of mannosidase I, has been synthesized in 13 steps from chlorobenzene by microbial oxygenation with *Pseudomonas putida* 39D and stereocontrolled peripheral functionalization of *cis*-3-chlorocyclohexa-3,5-dienediol **3**.

General glycosidase inhibition and processing of glycoproteins continue to attract attention because of their role in viral infection,[1] including HIV, the onset of tumour growth[2] and the immune system response.[3] Aza sugars of type **2** and their five-membered ring cousins are the subject of vigorous synthetic and medicinal research that address specific glycosidase inhibition activities.



2, mannojirimycin **1**, kifunensine **3** **4**

Kifunensine **1**, a unique oxamide derivative of mannojirimycin **2**, has shown promising immunomodulatory activity in α-mannosidase inhibition.[4–6] Its structure combines the aspects of oxygenated indolizidine alkaloids of the castanospermine type with that of the aza sugars. It is, therefore, not surprising that this compound has been shown to be a potent and specific inhibitor of mannosidase I, and of the processing of viral glycoproteins of the influenza in Madin–Darby canine kidney cells.[6] Its isolation from actinomycete *Kitasatosporia kifunense* No. 9482[5] was followed by structure determination[4] and a total synthesis, which included the preparation of its C-8a epimer, from D-mannosamine.[7a,b]

Our efforts in the utilization of *cis*-cyclohexadienediols[8] of type **3**,‡ derived by microbial oxidation of aromatic compounds with *Pseudomonas putida* 39D,[10] has taken us to the point of functionalization of the cyclohexadiene with an *exo*-located nitrogen atom (amino-conduritols)[11] or *endo*-placed nitrogen (aza sugars).[12] Kifunensine appeared to be an ideal target to effectively demonstrate the advantages of the mandate that uses biocatalytic oxidation of aromatics in synthesis.[13]

Our initial goal focussed on the introduction of the oxalamide ring onto the mannojirimycin derivative **7**, accessible from the diol **3** in seven steps (Scheme 1).§,[12]

Numerous attempts at the conversion of this compound into kifunensine *via* its amidine **7b** or the *N*-acyl lactam **7c** failed.¶

Ozonolysis of vinyl halides of type **6** may be controlled and leads to either five- or six-membered ring lactones or lactams, depending on the nature and the oxidation state of peripheral substituents.[12] Thus, the azido lactone **8** or lactam **7** can be attained selectively depending on functionalization of the 4-hydroxy group in **6**. Compound **8** was easily converted into the mannosamine derivative **9** and, therefore, provided for the intersection of Hashimoto's intermediate **10**[7b] *via* adjustments in the peripheral functionalization in the diol **3** obtained from chlorobenzene.

Thus, the lactone **8**, obtained by ozonolysis of **6b** (O_3/MeOH–H_2O/$NaHCO_3$/−78 °C) followed by reduction of the peroxide intermediate ($NaBH_4$/$CeCl_3$/−20 °C, crude yield 90–95%) and deprotection of the silyl group (Bu_4NF/dichloroethane/reflux, yield 70%), was transformed to the diacetonide **9** (30%) by refluxing in dimethoxypropane/dichloroethane/CSA (cat.) and reduction ($LiAlH_4$/Et_2O/RT/2 h). The introduction of the oxalate unit (MeOH/dimethyl oxalate/reflux) followed by methanolic ammonia treatment (10 min) furnished Hashimoto's intermediate **10** (55%), which was cyclized under the reported conditions to **11**§,[7b] (oxidation of the primary alcohol to the aldehyde and cyclization in methanolic ammonia). Deprotection in 75% trifluoroacetic acid gave (+)-kifunensine **1**, $[\alpha]_D^{25}$ +54 (*c* 0.066, H_2O) [lit.,[4] +58 (*c* 0.1, H_2O), identical with an authentic sample kindly furnished by Dr. H Kayakiri and M. Shirahashi].‖

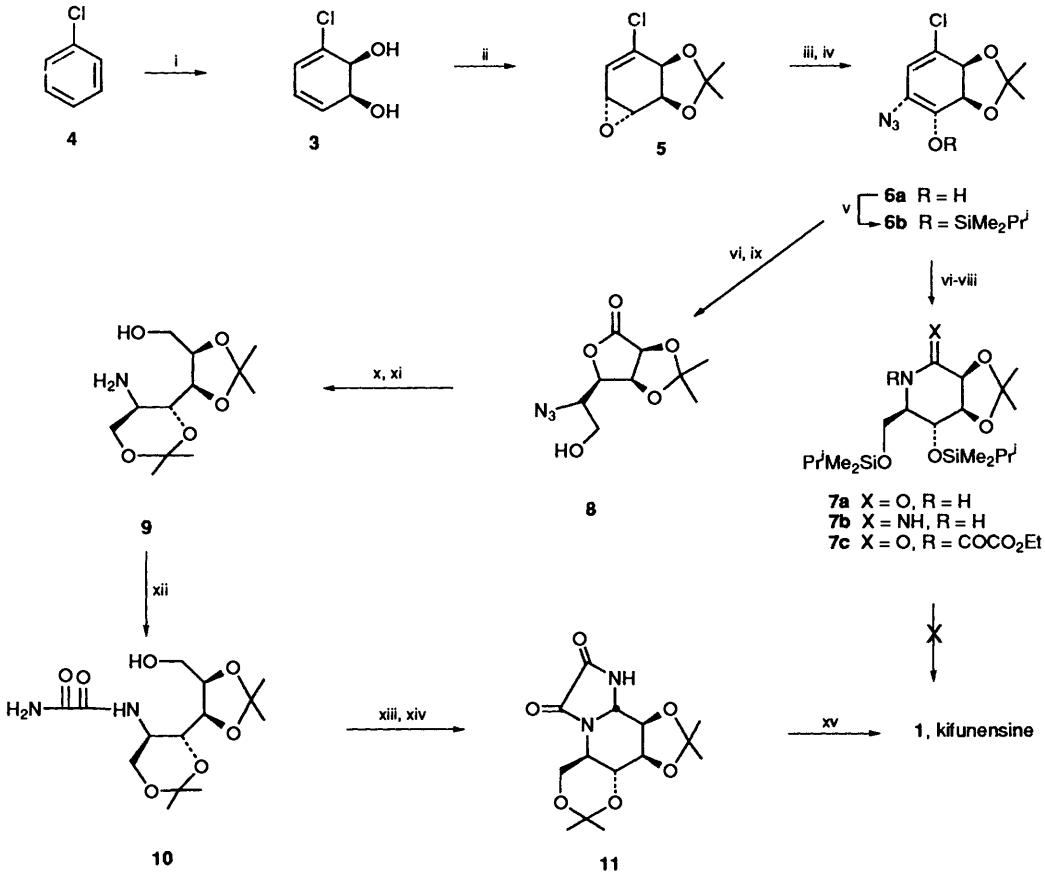
In summary, the synthesis of the bis-acetonide **11** has been accomplished in 12 steps from an achiral source, chlorobenzene. This feat compares favourably with the published synthesis, in which most stereocentres are already present in the starting mannosamine. Further improvements in the preparation of kifunensine will focus on a more direct transformation of **8** to **10** and will be reported in the near future.

Experimental

To a solution of compound **6b** (605 mg, 1.75 mmol) in methanol/7% water (150 cm^3), sodium hydrogen carbonate (735 mg, 8.75 mmol) was added; the mixture was cooled to −78 °C and ozonized until a blue colour persisted. The excess of ozone was removed with a stream of nitrogen at −78 °C. The temperature was raised to −20 °C and then $CeCl_3 \cdot 7H_2O$ (652 mg, 1.75 mmol) was added followed by $NaBH_4$ in portions (65 mg, 1.75 mmol, several times); the reaction was monitored by TLC (hexane–ethyl acetate, 8:2). When the reduction was complete (single spot by TLC, R_f 0.375 hexane–ethyl acetate, 3:1) a large excess of $NaBH_4$ was added and the reaction was quenched with water and citric acid at pH 6; the mixture was then stirred at room temp. for 1 h whereupon it was extracted with Et_2O (×3) and EtOAc (×2). The combined organic extracts were evaporated and the residue taken up in CH_2Cl_2

† Recipient of the American Cyanamid Faculty Research Award, 1992.
‡ For the experimental details connected to the medium scale (30–50 g) preparation of **3** and similar compounds see ref. 9.
§ H. Luna, Ph.D. Thesis, 1991, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.
¶ The chemistry associated with these transformations will be reported in an upcoming full paper.

§ The bisacetonide **11** displayed $[\alpha]_D^{25}$ −62 (*c* 0.2, $CHCl_3$) in comparison with −68 (*c* 0.3, $CHCl_3$) we recorded for an authentic sample kindly furnished by Dr. H. Kayakiri and M. Shirahashi. The original value[7a] was recorded in MeOH.
‖ [α] Values are recorded in units of 10^{-1} deg cm^2 g^{-1}.



Scheme 1 i, *Pseudomonas putida* 39D; ii, dimethoxypropane, TsOH cat., CH_2Cl_2, then *m*-chloroperbenzoic acid; iii, LiCl, ethyl acetoacetate, tetrahydrofuron; iv, NaN_3, dimethylformamide; v, Pr^iMe_2SiCl, imidazole, CH_2Cl_2; vi, O_3, MeOH–H_2O, $NaHCO_3$, −78 °C, then $NaBH_4$, $CeCl_3$; vii, Pr^iMe_2SiCl, 1,8-diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2; viii, H_2, Pd–C, MeOH; ix, Bu_4NF, (CH_2Cl); x, DMP, camphorsulfonic acid cat., $(CH_2Cl)_2$; xi, $LiAlH_4$, Et_2O; xii, $(MeO_2C)_2$, MeOH, then NH_3; xiii, CrO_3·2py, CH_2Cl_2; xiv, NH_3–MeOH; xv, 75% aq. CF_3CO_2H

and the solution washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to afford the crude azido alcohol (620 mg, 95%), shown to be 90% pure by ^1H NMR. To a solution of this crude azido alcohol (450 mg, 1.2 mmol) in 1,2-dichloroethane (20 cm^3) was added, at 0 °C, $Bu_4NF·3H_2O$ (454 mg, 1.44 mmol) and the mixture was stirred 1 h until it reached room temp. It was then stirred at reflux for 20 h, quenched at room temp. with water and extracted with CH_2Cl_2 (3 times). The combined organic layers were dried (Na_2SO_4), and evaporated under reduced pressure to give the crude lactone which was purified by flash chromatography (silica gel, hexane/ethyl acetate, 1 : 1) to afford the pure lactone **8** (205 mg, 70%); R_f 0.45 (hexane/ethyl acetate, 1 : 1); 115–116 °C (hexane, CH_2Cl_2); $\nu_{max}(CHCl_3)/cm^{-1}$ 3500br, 2105 and 1795; $\delta_H(CDCl_3)$ 4.91 (dd, *J* 5.1, 3.3,* 1 H), 4.86 (d, *J* 5.1, 1 H), 4.46 (dd, *J* 9.7, 3.3, 1 H), 4.04 (m, 1 H), 3.90 (m, 2 H), 1.94 (t, OH), 1.50 (s, 3 H) and 1.44 (s, 3 H); $\delta_C(CDCl_3)$ 173.5, 114.5, 76.06, 75.9, 62.1, 61.3, 26.8 and 25.9; *m/z* (CI) (rel. intensity) 244 (M + 1, 4), 228 (16), 216 (100), 186 (40) and 174 (30) (Found: C, 44.5; H, 5.4; N, 17.3. Calc. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.5; N, 17.3%).

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* *J* Values are recorded in Hz.

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