Synthesis of self-immolative glucuronide spacers based on aminomethylcarbamate. Application to 5-fluorouracil prodrugs for antibody-directed enzyme prodrug therapy

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The synthesis of three novel potential glucuronide-based prodrugs for antibody-directed enzyme prodrug therapy (ADEPT) is described. These prodrugs were designed to be activated at the tumour site by β-glucuronidase to afford the corrresponding anticancer agent, 5-FU. The structural pattern of these compounds includes a self-immolative spacer between the glucuronyl residue and the N^1 of 5-FU. Three types of spacers have been elaborated which, after enzymic hydrolysis, spontaneously decompose to deliver an unstable N^1 aminal 5-FU derivative and, from there, the cytotoxic drug. All potential prodrugs were stable and proved to be excellent substrates of *E. coli* in *in vitro* experiments.

Introduction

Antibody-directed enzyme prodrug therapy (ADEPT) is a new strategy of cancer chemotherapy based upon a selective delivery of drugs to the tumour cell surface using monoclonal antibody enzyme conjugates. After localization of this conjugate to its target cell, a non-toxic prodrug is administered which is expected to be converted into the potent anti-tumour agent.[1] Many studies aimed at testing the feasibility of this approach have been carried out using a range of prodrug/enzyme systems.[2] Among those, glucuronide-based prodrugs such as HMR 1826 are particularly interesting[3,4] as they can be selectively activated at the target site using a fusion protein[5] consisting of the human β-glucuronidase combined with the humanized Fab (antibody fragment) of the anti-CEA Mab (monoclonal antibody) BW 431. Not only can a dramatic increase of doxorubicin concentration in tumour cells be obtained by this protocol but, more recently, it has also been demonstrated that there is a high level of β-glucuronidase in necrotic tissues which allows, in this case, a site-selective activation of the glucuronyl prodrug according to a prodrug monotherapy protocol (PMT).[6]

In continuation and as an extension of our previous work on doxorubicin prodrugs,[4] our next interest was to design prodrugs of the inhibitor of thymidilate synthetase, 5-fluorouracil (5-FU). Such a drug is clinically useful for the treatment of solid tumours and remains the standard chemotherapy for colorectal cancers, although the response rate is rather low.[7] A great deal of research has been directed to improving the activity by increasing the concentration of 5-FU at the tumour site and the protection of non-target tissues from toxic effects. To address this problem, selective delivery of 5-FU to tumours *versus* normal tissues by targeted antibody-microbial cytosine deaminase conjugates (ADEPT) has been successfully reported[8] in tumour-bearing mice. However, it remains to be seen whether these impressive results can be translated into clinical trials without generating an immune response.[9] Obviously, a similar approach taking advantage of glucuronide prodrugs and also the above fusion protein, may escape this problem and at the same time may be more selective for colon cancer, since carcinoembryonic antigen (CEA) is widely expressed in the colorectal tractus. With this goal in mind, and applying a similar strategy to the doxorubicin tripartate-prodrugs, but with the less reactive amine-containing 5-FU, the first purpose was to elaborate a spacer between the drug and the glucuronyl residue which spontaneously releases the drug after enzymic cleavage. As a result, we would like to report the synthesis of two new types of tripartate glucuronide-based prodrugs as well as their enzymic behaviour in the presence of *E. coli* β-glucuronidase. The rationale for the first prodrug structure relied on the known fast enzymic hydrolysis of glycosides of *p*-nitrophenyl carbamate which, through a 1,6-elimination, gives an unstable carbamic acid that decomposes to afford a free amino group.[4,10] We hypothesized that if this amino group is linked to the amine-containing drug *via* a methylene spacer (aminomethylcarbamate), this would release the drug according to the mechanism of decomposition depicted in Chart 1. Following this general mechanism, which is closely related to a peptide cleavage-based mechanism of liberation of 5-FU,[11,12] it remains possible to consider two kinds of prodrugs differing at the α-side-chain level of the aminal moiety by the lack (or the presence) of a carboxy group, *i.e.* **1**, R = H and **2**, R = CO_2H, respectively. The additional carboxylic function in compound **2** may influence the lipophilicity of the starting prodrug and/or the kinetics of the release mechanism.

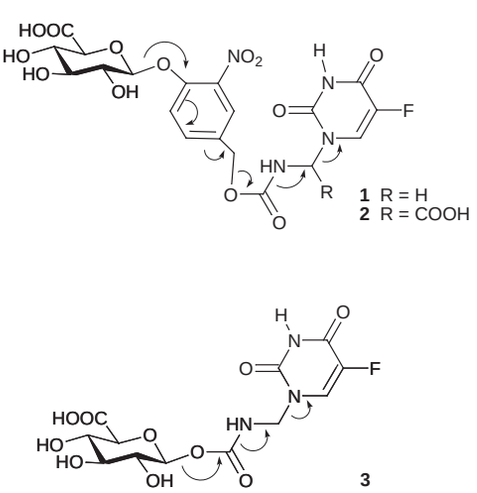


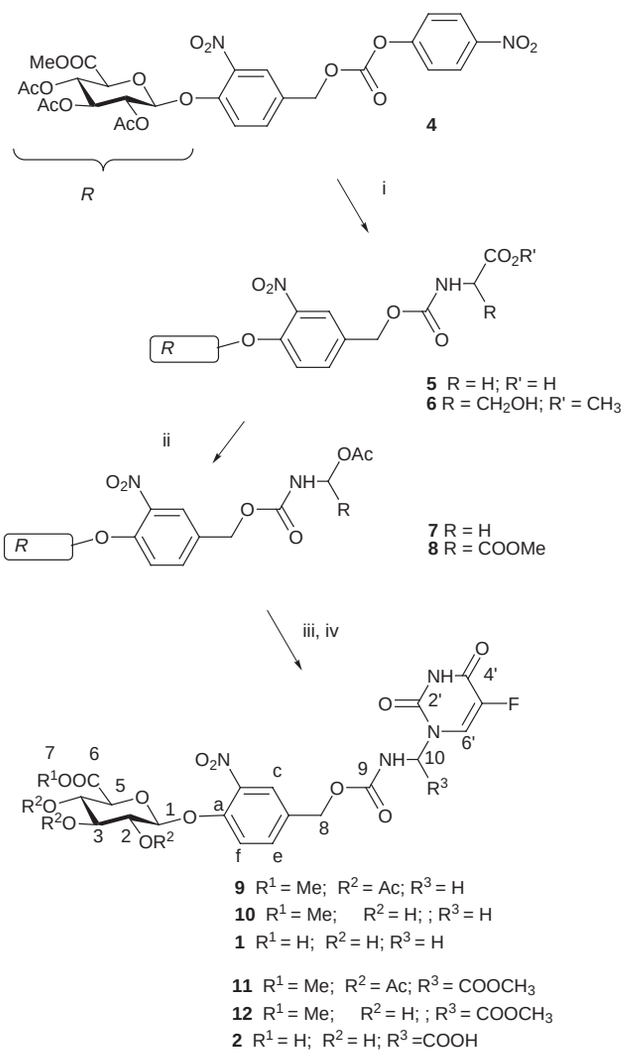
Chart 1

The shortest linkage between the glucuronyl carbamate and the amine-containing 5-FU, as present in the second tripartate type of prodrug **3**, is expected to decompose similarly.

Results and discussion

Synthesis

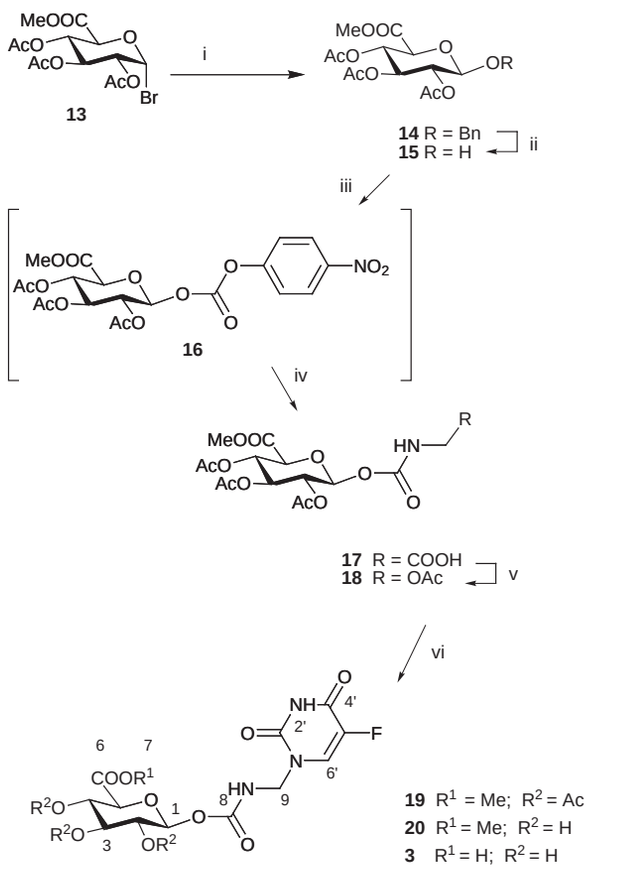
As shown in Scheme 1, the activated *O*-nitrophenyl glucuronyl



Scheme 1 *Reagents and conditions*: i) glycine (95%) or serine (OMe) (90%), Et_3N, DMF; ii) for **5**: $Pb(OAc)_4$, pyr, toluene (50%); for **6**: $Pb(OAc)_4$, AcOEt, 4 Å mol. sieves (61%); iii) 5-FU, Et_3N, DMF, rt (64% and 40% for **9** and **11** respectively); iv) a) MeO^-Na^+/MeOH, b) NaOH aq. (2 M), THF–water = 1 : 1 (72% and 23% for **1** and **2** respectively).

derivative **4**[4] was condensed with the amino acids glycine and serine methyl ester (Et_3N, DMF, rt, 4 h) to give the carbamates **5** (95%) and **6** (90%), respectively. These *N*-amino acid carbamate derivatives were next transformed into their α-acetoxy derivative by oxidative decarboxylation. Compound **5** was oxidized with lead tetraacetate to give **7** [$Pb(OAc)_4$, pyridine, toluene, THF, 50%],[13] whereas compound **6,** treated following Apitz and Steglich conditions [$Pb(OAc)_4$, 4 Å mol. sieves, EtOAc],[14] gave **8** (61%). The heterocyclic base 5-FU was finally condensed with acetoxymethylcarbamate derivatives **7** and **8** (Et_3N, DMF, rt) to give compound **9** (64%) and **11** (40%), respectively. Deprotection was carried out in two steps: removal of the acetyl groups by Zemplen transesterification (MeONa–MeOH), giving **10** or **12**, followed by cleavage of the methoxycarbonyl group (2M aq. NaOH in THF–water, 1 : 1) to afford the 5-FU prodrugs **1** (72%) and **2** (23%).

The synthesis of prodrug **3** required us to control the β-configuration of the carbamoyl linkage at the anomeric centre. Preliminary experiments to evaluate the direct anomeric activation of methyl 2,3,4-tri-*O*-acetylglucuronate by formation of a *p*-nitrophenyl carbamate with *p*-$NO_2C_6H_4COCl$ at 0 °C or at rt in THF or DMF were disappointing giving, in all events, a mixture of α- and β-anomers. Anticipating that the β-carbonate derivative **16** could be selectively obtained from the corresponding β-OH anomer, preparation of methyl 2,3,4-tri-*O*-acetyl-β-D-glucuronate **15** was attempted from the corresponding β-benzyl glycoside **14**. Indeed, in the glucose series, Kolar *et al.*[15] have shown that hydrogenolysis of a β-benzyl glucoside with 10% Pd/C affords the reductive analogue with retention of configuration. This means that mutarotation proceeds relatively slowly in the reaction medium. Therefore (Scheme 2) the benzyl



Scheme 2 *Reagents and conditions*: i) $AgCO_3$, BnOH, C_6H_6 (44%); ii) H_2, Pd/C, THF; iii) $ClCO_2C_6H_4NO_2$, Et_3N, THF; iv) Et_3N, THF, $^+H_3NCH_2COO^-$ Et_3N, DMF (70%); v) $Pb(OAc)_4$, pyr–toluene–THF (52%); vi) 5-FU, Et_3N, DMF (57%); vii) a) MeO^-Na^+, MeOH b) NaOH aq. (2 M), THF–water = 1 : 1 (77%).

glycoside **14** (44%) was prepared from the bromo-sugar **13**[16] under Koenigs–Knorr conditions with benzyl alcohol in the presence of silver carbonate as the catalyst. It must be noted that other silver salts or mercury salts were less efficient. Then, as expected, hydrogenolysis of **14** (Pd/C, H_2, THF) gave **15**, which was not isolated but was directly treated by addition of *p*-$NO_2C_6H_4COCl$ in the presence of triethylamine at rt to afford **16**. Since the carbonate **16** appeared to be very unstable during work-up and chromatography, direct addition of glycine to the crude solution of **16** was considered. Actually, this one-pot strategy allowed isolation of the expected glycinylcarbamate **17** from **14** in rather good yield (84%).

Oxidation of **17** with lead tetraacetate, as described in the case of **5**, furnished **18** (52%) which, after condensation with 5-FU (DMF, Et_3N), gave **19** (57%). Sequential deprotection of **19**, as described for **9** and **11** (*vide supra*) led to **20**, and further to prodrug **3**.

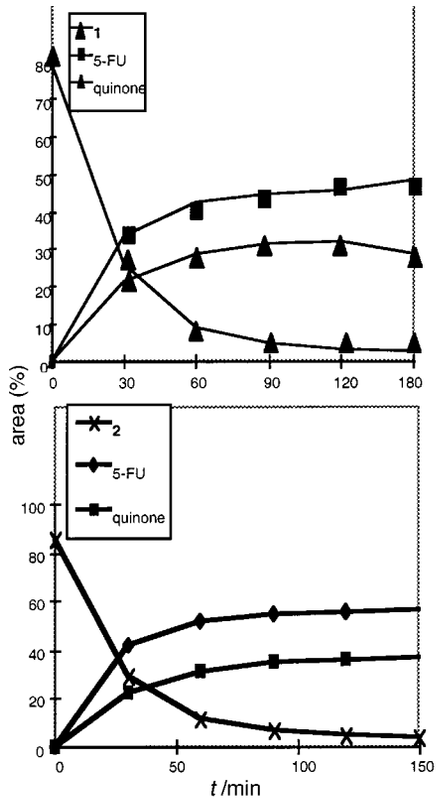


Fig. 1 Kinetics of drug release for the systems **1** (or **2**) + glucuronidase. *Conditions*: [prodrug] 250 μg ml^{-1}, [β-Glu *E. coli*] 0.05 μg ml^{-1}, 37 °C, phosphate buffer 0.02 M, pH 7.2.

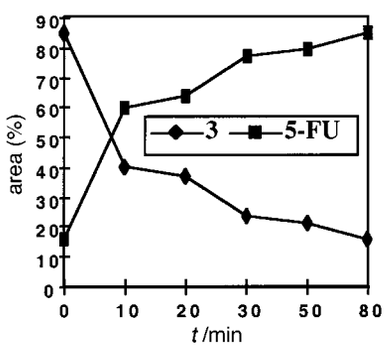


Fig. 2 Kinetics of drug release for the system **3** + β-glucuronidase. *Conditions*: [prodrug] 250 μg ml^{-1}, [β-Glu *E. coli*] 25 μg ml^{-1}, 37 °C, phosphate buffer 0.1 M, pH 7.0.

Stability

The stability of prodrugs **1**, **2** and **3** has been examined by their incubation at concentrations of 100 or 250 μg ml^{-1} in phosphate buffer (0.1 M, pH 4.4 and 0.02 M, pH 7.2) and in plasma, respectively. Interestingly, more than 90% of each prodrug was recovered after 400 min under both conditions.

Kinetics of drug release

When **1** and **2** are incubated with β-glucuronidase (*E. coli*, 0.05 μg ml^{-1} at 37 °C in phosphate buffer, pH 7.2), rapid hydrolysis of the glucuronyl moiety occurred with production of 5-FU (Fig. 1). From HPLC analysis, an enzyme-catalysed half-life of 20 min was observed for prodrugs **1** and **2**. These results show that a rapid release of 5-FU from prodrugs can easily be observed in both cases without significant differences in the kinetics. In the case of prodrug **3** (*E. coli*, 25 μg ml^{-1} at 37 °C in phosphate buffer, pH 7.0), we observed a half-life of 16 min (Fig. 2).

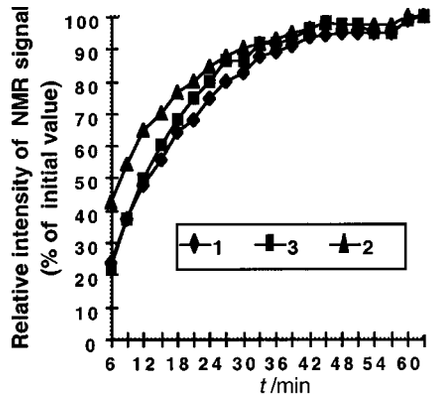


Fig. 3 Kinetics of drug release in the systems **1**–**3** + β-glucuronidase as measured by ^{19}F NMR analysis. *Conditions*: [prodrug] 5×10^{-3} M, [β-Glu β, beef liver] 5000 units ml^{-1}, 35 °C, PBS pH 7.4.

Kinetics of drug release followed by NMR analysis of ^{19}F (Fig. 3)

The kinetics of drug-release from prodrugs **1**, **2** and **3** has been determined by ^{19}F NMR analysis. Prodrugs were incubated in phosphate-buffered saline (PBS, pH 7.4) at 35 °C and at a concentration of 5×10^{-3} M. The spectra were registered on a 470.2 MHz Varian Unity 500 apparatus after addition of 5000 units ml^{-1} of β-glucuronidase (β_1 beef liver 560 000 units g^{-1}); a total of 20 spectra were recorded (1 every 3 min).

As depicted in Fig. 3, the kinetics of formation of the drug 5-FU from prodrugs **1**, **2** and **3** could be determined by integration of the ^{19}F signal of 5-FU at 2164 au which is sufficiently different from the ^{19}F NMR signal present in the starting prodrug at 135 au for **1**, 1299 au for **2** and 1270 au for **3**.

From this study, it appears that the kinetics of hydrolysis of the prodrugs can be classified as prodrug **3** > prodrug **1** > prodrug **2**.

Cytotoxicity

Prodrugs **1** and **2** were tested for cytotoxicity measured against LoVo (Human colon cancer cell line) cells using sulforhodamine B (SRB) assay. Compounds **1** and **2** exhibited IC_{50}-values of 75 μM and 51 μM, respectively. After enzymic hydrolysis with *E. coli* β-glucuronidase, an increased cytotoxicity was observed for both prodrugs with IC_{50}-values (≈12 μM) very close to that of the parent-drug 5-FU (8.5 μM).

Conclusions

From these results, it appears that the three prodrugs **1**–**3** are stable in plasma and, under enzymic cleavage of the glucuronyl moiety by *E. coli* glucuronidase, a rapid and efficient release of 5-FU is observed. We intend that investigation will be made on N^3-glucuronide-based prodrug derivatives of 1-(tetrahydro-2-furyl)-5-FU Ftorafur or other analogues to find out whether with these N^3-prodrugs, detoxification is much more pronounced than in the case of N^1 prodrugs **1**–**3**. Such a relatively low rate of detoxification (≈factor 6) with the latter is probably insufficient for relevant applications in ADEPT and, in that sense, any improvement in detoxification rate will be beneficial.

Experimental

Mps were taken on a Reichert apparatus or on a Koffler Bench and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg dm^{-1}, and the concentration (c) is given in g (100 ml^{-1}) in the specific solvent. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer (ν in cm^{-1}). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AC 300 spectrometer – chemical shifts δ in ppm and

J in Hz. NMR locants follow the scheme shown in structures **9**–**12**. Chemical ionization mass spectra (CI-MS; NH_3, positive-ion mode) and electronic impact mass spectra (EI-MS) were recorded on a Nermag R 10-10C spectrometer. Electrospray ionization mass spectra (ESI-MS) were acquired with a quadrupole instrument with a mass to charge (*m*/*z*) range of 2000. The Nermag R 10-10 mass spectrometer used was equipped with an analytical atmospheric pressure electrospray source. High-resolution (HR-MS) and fast-atom bombardment (FAB-MS) mass spectra were recorded on a Micromass ZAB2-SEQ spectrometer. Microanalyses were performed on a Perkin-Elmer 2400CHN microanalyser. UV-visible spectra were measured on a Varian-Cary 3E spectrophotometer. LoVo cells were obtained from ATCC (Rockville, MD, USA).

N-{4-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-uronate]-3-nitrobenzyloxycarbonyl}glycine 5

To a solution of compound **4**[4] (4 g, 6.15 mmol) in anhydrous THF were added glycine (460 mg, 6.15 mmol) and Et_3N (1.72 ml) and the reaction mixture was stirred at rt for 18 h. Then DMF was evaporated under reduced pressure (1 mmHg) and the residue purified by flash column chromatography (CH_2Cl_2–MeOH–AcOH, 94:5:1) to give compound **5** (3.42 g, 95%) as a gum ($[\alpha]_D^{20}$ +3 (*c* 1.2, MeOH); $\nu_{max}(CDCl_3)/cm^{-1}$ 3448, 1759, 1603, 1538, 1236; δ_H(300 MHz; $CDCl_3$) 7.78 (s, 1H, H-c), 7.52 (d, 1H, *J* 9.1, H-e), 7.34 (d, 1H, *J* 8.6, H-f), 5.65 (1H, signal exchangeable with D_2O, NH), 5.36–5.25 (m, 4H, H-1, -2, -3, -4), 5.08 (s, 2H, ArCH_2), 4.28 (d, 1H, *J* 8.4, H-5), 3.94 (signal exchangeable with D_2O, 1H, CO_2H), 3.80 (s, 2H, CH_2), 3.73 (s, 3H, CO_2CH_3), 2.11 and 2.08 (2s, 3H and 6H, OAc); *m*/*z* (CI) 604 $[M + NH_4]^+$.

N-{4-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-uronate]-3-nitrobenzyloxycarbonyl}serine methyl ester 6

To a solution of compound **4** (1 g, 1.5 mmol) in DMF (40 ml) were added methyl serinate (239 mg, 2 mmol) and Et_3N (0.43 ml). The reaction mixture was stirred for 18 h at rt, then evaporated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2–MeOH, 95:5) to give compound **6** (880 mg, 90%) (Found: C, 47.61; H, 4.85; N, 4.53. $C_{25}H_{30}N_2O_{17}$ requires C, 47.62; H, 4.80; N, 4.44%); $[\alpha]_D^{20}$ +14 (*c* 1, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 3434, 1757, 1623, 1538, 1373, 1240; δ_H(300 MHz; $CDCl_3$) 7.78 (s, 1H, H-c), 7.51 (d, 1H, *J* 8.6, H-e), 7.33 (d, 1H, *J* 8.6, H-f), 5.91 (d, 1H, *J* 7.9, NH), 5.34–5.20 (m, 4H, H-1, -2, -3, -4), 5.08 (s, 2H, H_2-8), 4.42–4.38 (m, 1H, H-10), 4.23 (d, 1H, *J* 8.8 Hz, H-5), 4.01–3.85 (ABX system, J_{AB} 27.7, J_{AX} 8.2, J_{BX} 13.9, 2H, CH_2OH), 3.74 (s, 3H, CO_2CH_3), 3.72 (s, 3H, CO_2CH_3), 2.70 (s exchangeable with D_2O, 1H, OH), 2.08 and 2.03 (2s, 3H and 6H, OAc); δ_C(62.5 MHz; $CDCl_3$) 170.7, 169.8, 169.1 and 166.5 (*C*OCH_3, 2 × *C*O_2CH_3), 155.5 (C-9), 148.4 (C-a), 140.6 (C-d), 133.1 (C-e), 132.3 (C-b), 124.2 (C-c), 119.4 (C-f), 99.2 (C-1), 72.1, 70.8, 69.9 and 68.4 (C-2, -3, -4, -5), 64.8 (C-8), 62.5 (CH_2OH), 55.8 (C-10), 52.8 and 52.4 (2 × O*C*H_3), 20.2 ($COCH_3$); *m*/*z* (CI) 648 $[M + H]^+$.

N-{4-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-uronate]-3-nitrobenzyloxycarbonyl]acetoxymethylamine 7

To derivative **5** (3.43 g, 5.9 mmol) in a mixture of anhydrous toluene–THF (92 ml; 1:3 v/v) under argon were added pyridine (0.5 ml) and $Pb(OAc)_4$ (3.26 g, 7.4 mmol). The reaction mixture was refluxed for 4 h, then filtered on a Celite pad. The resulting solution was evaporated under reduced pressure to give a solid, which was purified by flash column chromatography (cyclohexane–acetone, 2:1) to give *compound* **7** (1.76 g, 50%) as a gum (Found: C, 48.14; H, 4.93; N, 4.43. $C_{24}H_{28}N_2O_{16}$ requires C, 48.01; H, 4.70; N, 4.67%); $[\alpha]_D^{20}$ +8 (*c* 0.4, $CDCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3447, 1757, 1539, 1369; δ_H(300 MHz; $CDCl_3$) 7.80 (s, 1H, H-c), 7.55 (d, 1H, *J* 9.0, H-e), 7.35 (d, 1H, *J* 8.6, H-f), 5.36-5.11 (m, 8H, H-1, -2, -3, -4, H_2-8, -10), 4.26–4.21 (m, 1H, H-5), 3.73 (s, 3H, CO_2CH_3), 2.11 and 2.05 (2s, 3H and 9H, OAc); *m*/*z* (CI) 618 $[M + NH_4]^+$.

2-Acetoxy-*N*-{4-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-gluco-pyranosyl)uronate]-3-nitrobenzyloxycarbonyl}glycine methyl ester 8

Compound **6** (200 mg, 0.32 mmol) was dissolved in anhydrous EtOAc (8 ml), then $Pb(OAc)_4$ (211 mg, 1.5 equiv.) was added, followed by 4 Å molecular sieves. The reaction mixture was heated at reflux for 3 h. After cooling of the mixture to rt, 20% aq. citric acid (10 ml) was added. After stirring for 10 min, the two layers were separated. The organic layer was washed with 10% aq. NaCl and dried over $MgSO_4$. After filtration, evaporation gave a residue, which was purified by flash column chromatography (CH_2Cl_2–MeOH, 99:1) to afford *compound* **8** (127.5 mg, 61%) (Found: C, 47.49; H, 4.59; N, 4.55. $C_{26}H_{30}N_2O_{18}$ requires C, 47.42; H, 4.59; N, 4.25%); $[\alpha]_D^{20}$ +9 (*c* 1, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 3427, 1757, 1539, 1369, 1219; δ_H(250 MHz; $CDCl_3$) 7.80 (s, 1H, H-c), 7.53 (d, 1H, *J* 8.6, H-e), 7.35 (d, 1H, *J* 8.6, H-f), 5.97 (d, 1H, *J* 9.2, NH), 5.35–5.19 (m, 5H, H-1, -2, -3, -4, -10), 5.12 (s, 2H, H_2-8), 4.23–4.20 (m, 1H, H-5), 3.80 (s, 3H, CO_2CH_3), 3.72 (s, 3H, CO_2CH_3), 2.10 and 2.04 (s, 3H and 9H, OAc); δ_C(62.5 MHz; $CDCl_3$) 169.7, 169.0, 168.9, 167.6 and 166.4 (*C*OCH_3, 2 × *C*O_2CH_3), 155.0 (C-9), 148.6 (C-a), 140.9 (C-d), 133.1 (C-e), 131.9 (C-b), 124.4 (C-c), 119.8 (C-f), 99.4 (C-1), 72.2, 70.7, 69.8 and 68.4 (C-2, -3, -4, -5), 65.1 (C-8), 56.1 (C-10), 52.7 (2 × OCH_3), 20.2 ($COCH_3$); *m*/*z* (CI) 676 $[M + NH_4]^+$.

5-Fluoro-*N*-{4-*O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyr-anosyl)uronate]-3-nitrobenzyloxycarbonyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-ylmethylamine 9

5-FU (156 mg, 12 mmol) and Et_3N (1.67 μl, 12 mmol) were successively added to a solution of compound **7** (750 mg, 12.5 mmol) in anhydrous DMF (15 ml). After being stirred for 18 h at rt, the reaction mixture was evaporated under reduced pressure to give a residue, which was purified by flash column chromatography (CH_2Cl_2–MeOH, 98:2) to give *compound* **9** (536 mg, 64%) (Found: C, 45.63; H, 4.15; N, 8.41. $C_{26}H_{27}N_4O_{16}F$ requires C, 45.60; H, 4.13; N, 8.51%); $[\alpha]_D^{20}$ +7 (*c* 0.7, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 1760, 1727, 1538, 1368, 1237; δ_H(250 MHz; $CDCl_3$) 7.80 (s, 1H, H-c), 7.68 (d, 1H, *J* 5, H-6′), 7.52 (d, 1H, *J* 8.5, H-e), 7.35 (d, 1H, *J* 8.6, H-f), 6.78 (1H, s exchangeable with D_2O, NH), 5.37–5.23 (m, 4H, H-1, -2, -3, -4), 5.11 (s, 2H, H_2-8), 5.00 (d, 2H, *J* 6.4, H_2-10), 4.89–4.25 (m, 1H, H-5), 3.73 (s, 3H, CO_2Me), 2.11 and 2.05 (2s, 3H and 6H, OAc); δ_C(62.5 MHz; $CDCl_3$) 169.9, 169.3 and 166.7, (*C*OCH_3, C-6, -4′), 156.4 (C-9), 150 (C-2′), 148.8 (C-a), 142 (C-5′), 141 (C-d), 137.9 (C-b), 133.3 (C-e), 129 (C-6′), 124.6 (C-c), 119.6 (C-f), 99.4 (C-1), 77.3, 70.9, 70.1 and 68.6 (C-2, -3, -4, -5), 65.5 (C-6), 55.3 (C-10), 53.0 (OCH_3), 20.4 (*C*H_3CO).

5-Fluoro-*N*-{4-*O*-[methyl (β-D-glucopyranosyl)uronate]-3-nitrobenzyloxycarbonyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-ylmethylamine 10

To a cooled solution (−5 °C) of compound **9** (710 mg, 1.1 mmol) in MeOH (10 ml) was added sodium methoxide (65 mg, 1.65 equiv.). After being stirred for 5 h, the reaction was neutralized by addition of IRC 50 (H^+) Amberlite resin, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography (MeOH–CH_2Cl_2, 5:95 then 10:90), giving *compound* **10** (310 mg, 53%) as a gum (Found: C, 40.11; H, 4.55; N, 9.80. $C_{20}H_{21}N_4O_{13}F{\cdot}3H_2O$ requires C, 40.14; H, 4.55; N, 9.36%); $[\alpha]_D^{20}$ −41 (*c* 0.8, MeOH); ν_{max}(NaCl)/cm^{-1} 3377, 1676, 1538, 1353, 1065; δ_H(250 MHz, CD_3OD) 7.91 (d, 1H, *J* 5.9, H-6′), 7.89 (s, 1H, H-c), 7.59 (d, 1H, *J* 8.7, H-e), 7.38 (d, 1H, *J* 8.6, H-f), 5.20 (d, 1H, *J* 5.5, H-1), 5.11 (s, 2H, H_2-8), 4.99 (s, 2H, H_2-10), 4.13 (m, 1H, H-5), 3.76

(s, 1H, H-7), 3.60 (t, 1H, *J* 8.9, H-2), 3.55–3.48 (m, 2H, H-3, -4); δ_C(75 MHz, CD_3OD) 170.7 (C-6), 158.6 (C-9), 151.4 (C-2′), 150.6 (C-a), 142.7 (C-5′), 142.0 (C-d), 139.6 (C-4′), 134.5 (C-e), 132.8 (C-b), 130.8 (C-6′), 125.7 (C-c), 118.8 (C-f), 102.3 (C-1), 77.1, 76.7, 74.4 and 72.6 (C-2, -3, -4, -5), 66.4 (C-8), 55.8 (C-10), 53.0 (C-7); *m*/*z* (ES^+) 545 $[M + H]^+$, 567 $[M + Na]^+$, 583 $[M + K]^+$.

5-Fluoro-*N*-[4-*O*-(β-D-Glucopyranosyluronic acid)-3-nitrobenzyloxycarbonyl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-ylmethylamine 1

A solution of compound **10** (290 mg, 0.5 mmol) in THF–water (10 ml; 1:1) was cooled to −10 °C, and aq. NaOH (2 M; 0.920 ml) was added. The reaction mixture was stirred for 1 h at rt, then neutralized by addition of AcOH, and evaporated under reduced pressure to give a gum, which was purified by flash chromatography (CH_3CN–water, 9:1) to give *compound* **1** (191 mg, 72%) as a gum (Found: C, 43.10; H, 3.55; N, 10.25. $C_{19}H_{19}N_4O_{13}F$ requires C, 43.03; H, 3.61; N, 10.56%); $[\alpha]_D^{20}$ −44 (*c* 1, H_2O); ν_{max}(KBr)/cm^{-1} 3345, 3335, 1703, 1537, 1367; δ_H(300 MHz, D_2O) 7.81 (d, 1H, *J* 2.4, H-6′), 7.70 (s, 1H, H-c), 7.45 (d, 1H, *J* 7.7, H-e), 7.24 (d, 1H, *J* 7.9, H-f), 5.17–4.67 (m, 3H, H-1, -8, -10), 3.88-3.85 (m, 1H, H-5), 3.58 (m, 3H, H-2, -3, -4); δ_C(75 MHz, D_2O) 173.5 (C-6), 156.1 (C-9), 148.7 (C-2′), 147.9 (C-a), 139.7 (C-5′), 137.7 (C-d), 136.6 (C-4′), 132.8 (C-e), 131 (C-b), 129.0 (C-6′), 123.1 (C-c), 116.2 (C-f), 98.9 (C-1), 74.7, 73.7, 70.9 and 70.0 (C-2, -3, -4, -5), 64.0 (C-8), 53.5 (C-10); λ_{max}(H_2O)/nm 266 (ε 13 650); λ_{max}(0.1 M NaOH)/nm 266 (ε 13 175); *m*/*z* (ES^+) 531 $[M + H]^+$, 553 $[M + Na]^+$, 569 $[M + K]^+$.

N-{4-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-uronate]-3-nitrobenzyloxycarbonyl}-2-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)glycine methyl ester 11

To a solution of **8** (520 mg, 0.8 mmol) in anhydrous DMF (12 ml) were added 5-FU (100 mg, 0.8 mmol) and Et_3N (0.1 ml, 0.96 equiv.). The reaction mixture was stirred for 18 h at rt and then evaporated under reduced pressure to give a residue which was purified by flash column chromatography (CH_2Cl_2–MeOH, 97:3) to furnish *compound* **11** (226 mg, 40%) (Found: C, 45.49; H, 4.17; N, 7.20. $C_{28}H_{29}N_4O_{18}F$ requires C, 45.26; H, 4.08; N, 7.89%); $[\alpha]_D^{20}$ +11 (*c* 1, $CHCl_3$); ν_{max}($CDCl_3$)/cm^{-1} 3425, 1761, 1539, 1365, 1236; δ_H(300 MHz; $CDCl_3$) 7.79 (m, 1H, H-c), 7.65 (d, 1H, *J* 5, H-6′), 7.50 (m, 1H, H-e), 7.35 (m, 1H, H-f), 7.09 (m, 1H, NH), 5.78 (d, 1H, *J* 7.5, H-10), 5.37–5.07 (m, 6H, H-1, -2, -3, -4, H_2-8), 4.28 (m, 1H, H-5), 3.82 (s, 3H, CO_2CH_3), 3.70 (s, 3H, CO_2CH_3), 2.11 and 2.05 (2s, 3H and 6H, OAc); δ_C(62.5 MHz; $CDCl_3$) 170.2, 169.5, 169.4, 166.9 and 165.4 ($COCH_3$, $COOCH_3$), 157.1 (C-4′), 155.6 (C-9), 149.2 (C-a), 149.0 (C-2′), 142.2 (C-5′), 141.1 (C-d), 133.7 (C-e), 131.7 (C-b), 126.9 (C-6′), 124.9 (C-c), 119.9 (C-f), 99.6 (C-1), 72.6, 71.2, 70.3 and 68.8 (C-2, -3, -4, -5), 67.3 (C-10), 66.1 (C-8), 54.2 and 53.2 (2 × OCH_3), 20.6 ($COCH_3$); *m*/*z* (CI) 729 $[M + H]^+$.

N-[4-*O*-[Methyl (β-D-glucopyranosyl)uronate]-3-nitrobenzyloxycarbonyl]-2-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)glycinemethyl ester 12

To a solution of compound **11** (1 g, 1.37 mmol) in anhydrous MeOH (15 ml) was added MeONa (80 mg) portionwise. The mixture was stirred at −5 °C for 5 h. After neutralization with IRC 50S Amberlite ion-exchange resin (H^+), filtration and evaporation of solvent, the residue was purified by flash column chromatography (CH_2Cl_2–MeOH, 95:5) to give *compound* **12** (326 mg, 39%) (Found: C, 42.55; H, 4.07; N, 9.13. $C_{22}H_{23}N_4O_{15}F{\cdot}H_2O$ requires C, 42.59; H, 4.06; N, 9.03%); $[\alpha]_D^{20}$ −49 (*c* 1.3, MeOH); ν_{max}($CDCl_3$)/cm^{-1} 3527, 1733, 1535, 1361; δ_H(250 MHz, CD_3OD) 7.94 (d, 1H, *J* 5.9, H-6′), 7.86 (s, 1H, H-c), 7.60 (d, 1H, *J* 8.7, H-e), 7.37 (d, 1H, *J* 8.6, H-f), 6.23 (s, 1H, H-10), 5.22 (d, 1H, *J* 6.3, H-1), 5.14 (s, 2H, H_2-8), 4.13 (d, 1H, *J* 9.4, H-5), 3.81 (s, 3H, CO_2CH_3), 3.78 (s, 3H, CO_2CH_3), 3.70–3.50 (m, 3H, H-2, -3, -4); δ_C(75 MHz, CD_3OD) 170.9 and 167.7 (2 × CO_2CH_3), 160.1 (C-4′), 157.7 (C-9), 151.1 (C-2′), 150.6 (C-a), 141.9 (C-5′), 139.9 (C-d), 134.9 (C-e), 132.5 (C-b), 130.1 (C-6′), 125.9 (C-c), 118.8 (C-f), 102.2 (C-1), 76.9, 76.6, 74.3 and 72.6 (C-2, -3, -4, -5), 67.4 (C-10), 66.9 (C-8), 54.4 and 53.3 (2 × OCH_3); *m*/*z* (ES^+) 603 $[M + H]^+$, 625 $[M + Na]^+$.

N-[4-*O*-(β-D-Glucopyranosyluronic acid)-3-nitrobenzyloxycarbonyl]-2-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)glycine 2

To a solution of compound **12** (326 mg, 0.54 mmol) in a 1:1 mixture of THF–water (20 ml) at −10 °C was added 2 M aq. NaOH (0.914 ml). The reaction mixture was stirred at rt for 1 h, then neutralized with AcOH and evaporated under reduced pressure to give a residue, which was purified by flash column chromatography (CH_3CN–water–AcOH, 79.9:20:0.1) to give *compound* **2** (70 mg, 23%) as a gum (Found: C, 41.90; H, 3.22; N, 9.63. $C_{20}H_{19}N_4O_{15}F$ requires C, 41.82; H, 3.33; N, 9.75%); $[\alpha]_D^{20}$ −31 (*c* 1, H_2O); ν_{max}(KBr)/cm^{-1} 3689, 1715, 1541, 1385; δ_H(300 MHz, D_2O) 7.81–7.70 (m, 2H, H-c, -6′), 7.49–7.43 (m, 1H, H-e), 7.22–7.15 (m, 1H, H-f), 5.77 (s, 1H, H-10), 5.07–5.01 (m, 3H, H-1, H_2-8), 3.84 (br s, 1H, H-5), 3.55 (br s, 3H, H-2, -3, -4); δ_C(75 MHz, D_2O + 1 drop of dioxane) 176.2 and 171.1 (2 × COOH), 161.0 (C-4′), 158.0 (C-9), 150.9 (C-2′), 150.5 (C-a), 140.3 (C-5′), 139.5 (C-d), 135.5 (C-e), 132.1 (C-b), 130.6 (C-6′), 126.6 (C-c), 118.9 (C-f), 101.5 (C-1), 77.4, 76.3, 73.6 and 72.6 (C-2, -3, -4, -5), 69.1 (C-10), 66.8 (C-8); λ_{max}(H_2O)/nm 267 (ε 16 192); λ_{max}(0.1 N NaOH)/nm 265 (ε 15 066); *m*/*z* (ES^-) 573 $[M - H]^-$, 595 $[M - 2H + Na]^-$.

Methyl (benzyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate 14

To a solution of bromo compound **13** (350 mg, 0.96 mmol) in dry benzene (6 ml) were added, 4 Å mol sieves (400 mg), dry benzyl alcohol (2 equiv., 182.5 μl) and silver carbonate (364.7 mg, 1.5 equiv.) successively. The mixture was stirred at rt for 20 h and filtered. After evaporation of the organic solvents under reduced pressure, purification by flash column chromatography (cyclohexane–ethyl acetate, 3:1) gave *compound* **14** (164.7 mg, 44%), mp 138 °C (Found: C, 56.70; H, 5.91. $C_{20}H_{24}O_{10}$ requires C, 56.6; H, 5.7%); $[\alpha]_D^{20}$ −38.4 (*c* 1.09, $CHCl_3$); ν_{max}($CDCl_3$)/cm^{-1} 2956, 1757, 1249; δ_H(300 MHz; $CDCl_3$) 7.39–7.27 (m, 5H, ArH), 5.29–5.19 (m, 2H, H-2, H-3), 5.09 (dd, 1H, *J* 7.7, *J*′ 8.9, H-4), 4.94 and 4.63 (AB system, 2H, J_{AB} 12.3, H_2-8), 4.60 (d, 1H, *J* 7.6, H-1), 4.04–4.01 (m, 1H, H-5), 3.78 (s, 3H, H_3-7), 2.02–2.00 (m, 9H, OAc); δ_H(300 MHz, C_6D_6) 7.22–7.05 (m, 5H, ArH), 5.56–5.50 (t, 1H, *J* 9.5, H-4), 5.44–5.37 (m, 2H, H-2, -3), 4.72 and 4.34 (AB system, 2H, *J* 12.4, H_2-8), 4.31 (d, 1H, *J* 7.2, H-1), 3.78 (d, 1H, *J* 9.6, H-5), 3.33 (s, 3H, H_3-7), 1.68–1.60 (m, 9H, OAc); *m*/*z* (CI) 442 $[M + NH_4]^+$.

[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate] 4-nitrophenyl carbonate 16

A solution of compound **14** (2.66 g, 6.3 mmol) in dry THF (60 ml) was stirred under hydrogen atmosphere in the presence of Pd-on-charcoal (10%) for 5 h. The suspension was filtered on Celite, evaporated (to a volume of 20 ml), and cooled to 0 °C prior to the addition of *p*-nitrophenyl chloroformate (1.26 g) and Et_3N (0.96 ml). The resulting reaction mixture was stirred at rt for 18 h and evaporated under reduced pressure to give a residue. After column chromatography (cyclohexane–ethyl acetate, 2:1), *compound* **16** (406.9 mg, 13%) was obtained as a solid, mp 132 °C (Found: C, 47.87; H, 4.35; N, 2.77. $C_{20}H_{21}NO_{14}$ requires C, 48.10; H, 4.24; N, 2.80%); $[\alpha]_D^{20}$ −4.4 (*c* 0.34, $CHCl_3$); ν_{max}($CDCl_3$)/cm^{-1} 1758, 1521, 1341, 1223; δ_H(300 MHz; $CDCl_3$) 8.30 (d, 2H, *J* 6.9, ArH), 7.44 (d, 2H, *J* 7.2, ArH), 5.79 (d, 1H, *J* 6.5, H-1), 5.41–5.31 (m, 2H, H-3, -4), 5.21

(dd, 1H, *J* 6.4, *J′* 7.6, H-2), 4.31 (d, 1H, *J* 8.3, H-5), 3.77 (s, 3H, H_3-7), 2.12 and 2.06 (2s, 3H and 6H, OAc); *m/z* (CI) 517 $[M + NH_4]^+$.

N-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)-uronate]glycine 17

1. From compound 16. To a solution of compound **16** (1 g, 2 mmol) in dry DMF (30 ml), were added glycine (150.4 mg, 2 mmol) and Et_3N (0.3 ml) at rt. After stirring for 18 h, the DMF was evaporated under reduced pressure to give a residue, which was purified by flash column chromatography (EtOAc–CH_2Cl_2, 2:1, then 3:1 and AcOH 1%). *Compound* **17** (610 mg, 70%) was obtained as an oil (Found: C, 44.20; H, 5.34; N, 3.06. $C_{16}H_{21}NO_{13}$ requires C, 44.14; H, 4.86; N, 3.22%); $[a]_D^{20}$ +5.2 (*c* 0.27, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 3433, 1757, 1224; δ_H(300 MHz; $CDCl_3$) 6.70 (br, 1H, COOH), 6.00–5.98 (t, 1H, *J* 5.3, NH), 5.72 (d, 1H, *J* 7.9, H-1), 5.35 (t, 1H, *J* 9.4, H-3), 5.25–5.14 (m, 2H, H-2, -4), 4.21 (d, 1H, *J* 9.7, H-5), 4.00 (d, 2H, *J* 5.5, H_2-9), 3.73 (s, 3H, H_3-7), 2.10 and 2.03 (2s, 3H and 6H, OAc); *m/z* (CI) 453 $[M + NH_4]^+$, 436 $[M + H]^+$.

2. From compound 14. A solution of **14** (2.32 g, 5.47 mmol) was treated as for obtention of **16** (H_2, Pd/C, THF; then *p*-nitrophenyl chloroformate, Et_3N) but, after complete disappearance of the starting material, glycine (1.2 equiv.) and Et_3N (0.839 ml) were added to the reaction mixture and stirring was maintained overnight. Evaporation under reduced pressure followed by flash column chromatography (solvents as above) led to 2 g (84%) of title compound **17**.

Acetoxy-*N*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyloxy-carbonyl)uronate]methylamine 18

To compound **17** (290 mg, 0.67 mmol) dissolved in a mixture of THF–toluene 3:1 (15 ml) were added pyridine (0.054 ml) and $Pb(OAc)_4$ (0.37 mg, 1.25 equiv.). The reaction mixture was refluxed for 4 h and then allowed to reach rt. The reaction mixture was filtered and the filtrate evaporated under reduced pressure to give a residue which was purified by flash column chromatography (C_6H_{12}–EtOAc, 1:1), affording compound **18** (155 mg, 52%) as an oil $[a]_D^{20}$ +10 (*c* 1.13, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 1763, 1229; δ_H(300 MHz; $CDCl_3$) 6.19 (t, 1H, *J* 7.4, NH), 5.73 (d, 1H, *J* 7.8, H-1), 5.32 (t, 1H, *J* 9.2, H-3), 5.25 (t, 1H, *J* 9.5, H-4), 5.22–5.11 (m, 3H, H-2, H-9), 4.19 (d, 1H, *J* 9.6, H-5), 3.74 (s, 3H, H_3-7), 2.08, 2.04 and 2.03 (3s, 2 × 3H and 6H, OAc); δ_H(300 MHz, C_6D_6) 6.27 (t, 1H, *J* 7.4, NH), 5.85 (d, 1H, *J* 8.0, H-1), 5.61–5.47 (m, 3H, H-2, -3, -4), 4.95-4.92 (m, 2H, H_2-9), 3.86 (d, 1H, *J* 9.7, H-5), 3.26 (s, 3H, H_3-7), 1.65 and 1.55 (2s, 9H and 3H, OAc); δ_C(75 MHz; $CDCl_3$) 171.37, 169.77, 169.33, 166.69 and 166.14 (*C*OCH_3, C-6), 153.24 (C-8), 92.70 (C-1), 72.72, 71.63, 69.85 and 68.9 (C-2, -3, -4, -5), 65.99 (C-9), 52.94 (C-7), 20.75–20.46 (CO*C*H_3); *m/z* (CI) 467 $[M + NH_4]^+$.

N-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyloxycarbonyl)-uronate]-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-methylamine 19

5-FU (36.33 mg, 0.96 mmol) and Et_3N (0.04 ml) were successively added to a solution of compound **18** (129 mg, 0.29 mmol) in DMF (6 ml). The reaction mixture was stirred for 18 h at rt. After evaporation of DMF under reduced pressure, the residue, after purification by flash column chromatography (C_6H_{12}–EtOAc, 1:3), led to compound **19** (85.8 mg, 57%) (Found: C, 43.18; H, 4.24; N, 8.48. $C_{19}H_{22}N_3O_{13}F$ requires C, 43.94; H, 4.27; N, 8.09%); $[a]_D^{20}$ −13 (*c* 0.5, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 1757, 1669, 1230; δ_H(250 MHz; $CDCl_3$) 7.63 (d, 1H, *J* 5.25, H-6′), 5.66 (d, 1H, *J* 7.4, H-1), 5.34–5.05 (m, 3H, H-2, -3, -4), 4.94 (d, 2H, *J* 2.2, H_2-9), 4.18 (d, 1H, *J* 9.3, H-5), 3.70 (s, 3H, H_3-7), 2.06 and 2.01 (2s, 3H and 6H, OAc); δ_C(62.5 MHz; $CDCl_3$) 169.99, 169.84, 169.59 (*C*OCH_3), 167.47 (C-6), 154.98 (C-8), 150.23 (C-2′), 142.23 (C-5′), 138.43 (C-4′), 122.23 (C-6′), 93.12 (C-1), 72.78, 71.44, 70.21 and 69.05 (C-2, -3, -4, -5), 55.00 (C-9), 53.15 (C-7), 20.63, 20.53 and 20.46 (CO*C*H_3); *m/z* (CI) 537 $[M + NH_4]^+$.

N-[Methyl (β-D-glucopyranosyloxycarbonyl)uronate]-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methylamine 20

To a solution of compound **19** (700 mg, 1.35 mmol) in MeOH (15 ml) at −5 °C was added MeONa (120.2 mg, 1.6 equiv.). The mixture was stirred at the same temperature for 5 h and neutralized by addition of IRC 50S. After filtration, evaporation of the solvent and purification by flash column chromatography (CH_2Cl_2–MeOH, 9:1, then 8:2) *compound* **20** (250 mg, 49%) was isolated as a solid, mp 161 °C (Found: C, 37.92; H, 4.35; N, 10.34. $C_{13}H_{16}N_3O_{10}F\cdot H_2O$ requires C, 37.96; H, 4.41; N, 10.22%); $[a]_D^{20}$ −16 (*c* 0.43, MeOH); $\nu_{max}(CDCl_3)/cm^{-1}$ 3583, 1754, 1691, 1248; δ_H(300 MHz, CD_3OD) 7.88 (d, 1H, *J* 5.9, H-6′), 5.40 (d, 1H, *J* 8, H-1), 5.00 (s, 2H, H_2-9), 3.95 (d, 1H, *J* 9.4, H-5), 3.75 (s, 3H, H_3-7), 3.55–3.23 (m, 3H, H-2, -3, -4); δ_C(62.5 MHz, CD_3OD) 171.93 (C-6), 158.35 (C-8), 152.39 (C-2′), 144.06 (C-5′), 140.36 (C-4′), 131.91 (C-6′), 97.96 (C-1), 78.30, 78.20, 74.64 and 73.97 (C-2, -3, -4, -5), 56.67 (C-9), 53.98 (C-7); *m/z* (ES^+) 394 $[M + H]^+$, 416 $[M + Na]^+$.

N-(β-D-Glucopyranosyloxycarbonyluronic acid)-(5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-methylamine 3

A solution of compound **20** (200 mg, 0.51 mmol) in a 1:1 mixture of THF–water (8 ml) at −10 °C was stirred for 1 h in the presence of aq. NaOH (1 M, 0.614 ml). After neutralization with AcOH, the reaction solvents were evaporated and the residue obtained purified by flash column chromatography (MeCN–water, 90:10). Compound **3** (142 mg, 77%) was obtained as a solid, mp 165–170 °C (Found: C, 38.10; H, 3.65; N, 11.09. $C_{12}H_{14}N_3O_{10}F$ requires C, 38.00; H, 3.72; N, 11.08%); $[a]_D^{20}$ −4.17 (*c* 1.15, H_2O); $\nu_{max}(CDCl_3)/cm^{-1}$ 3447, 1716, 1575; δ_H(300 MHz, D_2O + a few drops of dioxane) 7.84 (d, 1H, *J* 5.7, H-6′), 5.32 (d, 1H, *J* 8.0, H-1), 4.96 (s, 2H, H_2-9), 3.72 (d, 1H, *J* 10, H-5), 3.46-3.38 (m, 3H, H-2, -3, -4); δ_C(75 MHz, D_2O) 175.96 (C-6), 157.19 (C-8), 151.30 (C-2′), 142.33 (C-5′), 139.25 (C-4′), 131.36 (C-6′), 95.77 (C-1), 77.05, 75.97, 72.46 and 72.30 (C-2, -3, -4, -5), 55.60 (C-9); $\lambda_{max}(H_2O)$/nm 267 (ε 4580); λ_{max}(0.1 M NaOH)/nm 270 (ε 3220); *m/z* (ES^-) 378 $[M - H]^-$, 400 $[M - 2H + Na]^-$, 417 $[M - 2H + K]^-$.

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