Note

A short and efficient transformation of rhamnose into activated daunosamine, acosamine, ristosamine and epi-daunosamine derivatives, and synthesis of an anthracycline antibiotic acosaminyl-ε-iso-rhodomycinone

Bernd Renneberg a, Yue-Ming Li a, Hartmut Laatsch a,*, Heinz-Herbert Fiebig b

a Institute of Organic Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany
b Oncotest GmbH, Am Flughafen 8-10, D-79110 Freiburg, Germany

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Abstract

3-Amino-2,3,6-trideoxyhexopyranoses are essential constituents of most anthracycline antitumour antibiotics. For an investigation of structure–activity relationships, the four diastereomeric amino sugars daunosamine, acosamine, ristosamine, and epi-daunosamine were synthesised in short and efficient syntheses starting from commercially available rhamnose. Several glycosyl donors were provided and their use was exemplified in the synthesis of acosaminyl-ε-iso-rhodomycinone. © 2000 Elsevier Science Ltd. All rights reserved.

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Many anthracycline antibiotics show strong antitumour activity [1,2]: the highest intracellular concentration of the drug is found in the nucleus, where it intercalates into the DNA double helix forming a ternary complex with DNA topoisomerase II [3], with consequent inhibition of replication and transcription. Because of the small difference between normal DNA and DNA from cancer cells, anthracyclines are generally very toxic also to hosts, and administration of the drug may cause serious side-effects such as nausea, vomiting, gastrointestinal toxicity, and most of all, cumulative cardiotoxicity (Scheme 1).

Anthracycline antibiotics like daunomycin or the ε-iso-rhodomycinone derivative D-788-6 (1a) consist of a tetracyclic chromophor and one or more sugar residues; the first moiety is usually an amino sugar, which may be the only sugar residue, or be part of an oligosaccharide chain. The configuration of the amino

* Corresponding author.
E-mail address: hlaatsch@gwdg.de (H. Laatsch).
have been synthesised from commercially available cheap D-mono-saccharides, however, with rather long reaction sequences or low yields [5]. The L-amino hexose 5a bearing xylo configuration is the C-3 epimer of L-daunosamine and has often been referred as 3-epi-daunosamine. It is not a naturally occurring isomer, and has been obtained most usually as a minor by-product during the synthesis of other amino sugars [6] (Scheme 2).

The first total syntheses of 5a were reported by Cheung [7] and Boivin [8], starting from \( \alpha \)-D-glucopyranoside. In our synthesis of amino sugar derivatives 2a–5a, L-di-O-acetyl-rhamnal (7) was used as the starting material. The latter was easily obtained in a 100 g scale, when a literature procedure for the synthesis of acetobromo sugars [9] and the reductive dehalogenation were optimised and performed in a one-pot-reaction. L-(+)-Rhamnose (6a) was first peracetylated and transformed into acetobromorhamnose (6b) using \( \text{PBr}_3 \) as the brominating agent and then reduced by a zinc–copper alloy giving the expensive L-(+)-di-O-acetyl-rhamnal (7) in more than 85% yield (Scheme 3).

Heating the aqueous suspension of 7 at 80 °C [10] delivered the hydrolysis product 8a which was used for the synthesis of acosamine (3a) and ristosamine (4a) derivatives. In contrast to the literature [11], addition of azide on 8a occurred with only moderate stereoselectivity, and both 3b and 4b were obtained in a ratio of merely 2.1:1. Silylation of the crude product with tert-butyl(dimethyl)silyl chloride–imidazole using DMF as the solvent afforded a mixture of \( \alpha \) and \( \beta \) anomers [12]; in dichloromethane or 1,2-dichloroethane, however, both 3c and 4c (84%) were obtained as pure \( \beta \) anomers [13]. After removal of the acetyl groups using \( \text{K}_2\text{CO}_3 \)–MeOH, the ob-

1 The condensation of \( \alpha \)-rhodomycinone (1b) with an \( \alpha \)-1-O-trimethylsilylated daunosamine donor, catalyzed by TMS-triflate, gave a complex mixture of products, from which \( \alpha \),\( \alpha \) and \( \alpha ,\beta \)-disaccharide as well as an \( \alpha \)-rhodomycin with \( \beta \)-glycosidic linkage have been isolated. As these side reactions could not be avoided in the case of daunosamine donor 2f by using the more stable \( \beta \)-1-O-tert-butyldimethylsilyl glycoside, we selected TBDMS as the protective group in the syntheses of other glycosyl donors as well.

sugar influences strongly the bioactivity of the drug: replacement of L-daunosamine (2a) with L-acosamine (3a) or L-ristosamine (4a) in anthracyclines produces analogues having similar antitumour activity but reduced cardiotoxicity [4]. In this paper we describe an optimised short synthesis for the L-amino sugars 2a–5a in an activated form useful for the glycosylation of anthracyclinones, as is exemplified in the coupling reaction of the acosamine derivative 3f with 1b affording acosaminyl-\( \alpha \)-iso-rhodomycinone (1c).

The amino sugars 2a–4a occur naturally as constituents of anthracycline antibiotics and
tained diastereomeric mixture of azides could be separated easily by column chromatography to yield pure 3d and 4d.

The result that azide addition on 8a gave both 3b and 4b, allowed a divergent synthesis of the diastereomeric L-amino sugars 2b and 5b in the same way. Thus, compound 7 was first converted in a Ferrer reaction to 8b (α and β anomer in a ratio of ~4:1), using zinc chloride in methanol as a catalyst [14], followed by deacetylation to afford compound 8c. Epimerisation of the 4-OH group by the Mitsunobu reaction furnished compound 9b with an axial 4-OH group. In the presence of hydrochloric acid in acetic acid and THF, the acetal group in 9b was cleaved, the resulting 9c was then subjected to azide addition. The resulting mixture was again protected with TBDMS without further purification and characterisation. After column chromatography, a colourless viscous oil was obtained. The 1H NMR spectrum showed that 2c and 5c were obtained in a ratio of about 1:2.4, very similar to the synthesis of 3c and 4c. Removal of the acetal groups delivered 2d and 5d.

Another facile access to the daunosamine series was possible starting from the acosamine derivative 3d: first, this was converted to the triflate 3h, followed by epimerisation at C-4 using AcOCl and sodium acetate. Although the reaction failed to give a high yield of 2e (43% overall yield from 3d), it is still more efficient than results reported previously [15].

The low stereo selectivity during azide addition on 8a or 9c is due to the small steric shielding of their 4-O-acetyl group, and reaction of benzoate 9a with azide should give much better yields of the 3-epi-daunosamine configuration, therefore. The hexenopyranoside 9a was obtained, however, by mesylation of 8c and subsequent nuclophilic substitution of the mesylate with caesium benzoate in only 39% yield; better results (80–90%) were obtained using the Mitsunobu reaction [16]: when the acetal group in 9a was cleaved with 1 N HCl in HOAc–THF and subsequently azide was added, only a single product was detectable by TLC. The 1-hydroxy group of this intermediate was finally protected as tert-butyl(dimethyl)silyl (TBSDMS) derivative. TLC analysis showed that again only one product was formed, which was further confirmed as the β anomer 5i.

From the 1H NMR spectrum of the final product it was not clear whether the compound had the lyxo or the xylo configuration. Removal of the benzoyl group with K2CO3 – CH3OH, however, gave a compound, the 1H NMR spectrum of which was not identical with that of the authentic lyxo-compound 2d, identifying the acetal as being the xylo derivative 5d with an axial azido group. This stereochemistry was further confirmed by NMR experiments with the derivative 5f: significant nuclear Overhauser effects (NOE) of NH with 1-H and 5-H allowed the assignment of the NHCOCF3 group as being axial, and a strong NOE between one 2-H signal and a weak NOE with the other allowed the assignment of chemical shifts of 2Hc and 2Hs. The 1-proton was assigned as being axial according to the coupling constant and also the strong NOE with the NH-group.

Reduction of all azides was done in the same way: when catalytic hydrogenation of 3d–4d was carried out in methanol in the presence of two equivalents of ethyl trifluoroacetate and triethyl amine, trifluoroacetylation of the amino group occurred simultaneously to give 3e and 4e, and the yield was 12% higher than using an improved literature procedure [17]. Protection of the 4-hydroxyl group as acetate or p-nitrobenzoate gave the acosamine derivatives 3f–3g and the ristosamine derivative 4g in good yields (>90%). With similar results, compound 2d was finally converted into the glycosyl donor 2f, and from 5d, 5f was obtained. All four protected L-amino sugars are versatile intermediates for the synthesis of anthracycline type anticancer antibiotics.

The glycosylation of ε-iso-rhodomycinone (1b) with the acosamine donor 3f proceeded smoothly in 1:1 dichloromethane–acetone in the presence of 1.6 equivalents of Me3Si–triflate and powdered molecular sieve (4 Å), when a temperature of about −35 °C was maintained. At lower temperature (−45 to −50 °C), the sparingly soluble 1b precipitated, and prolonged reaction time lead to side reactions, such as enhanced bisglycosylation, and decomposition of the glycosyl donor 3f.
cinon (1c) showed a very similar IC₇₀-pattern like doxorubicin (see Table 2). The colon cancer model (CCL HT29) and the lung cancer (LXF 529) were the most sensitive in contrast to the gastric cancer (GXF 251), lung cancer (LXF 629) and the melanoma (MEXF 462).

1. Experimental

General.—IR spectra (KBr pellet or neat) were recorded on Perkin–Elmer 297 spectrometer, ¹H NMR spectra were recorded on Varian VXR 200 (200 MHz), Bruker AM 300 (300 MHz); ¹³C NMR spectra were recorded on Varian VXR 200 (50.3 MHz), Bruker AM 300 (75.5 MHz), coupling constants J in Hertz, chemical shifts in δ related to tetramethylsilane as internal standard. Mass spectra were obtained on Varian MAT 73 and Varian 311A at 70 eV by electron impact. TLC plates (Silica Gel PF 254, E. Merck) and silica gel for column chromatography (0.05–0.2 mm, 70–270 mesh) was purchased from Macherey–Nagel & Co (Düren, Germany). L-(+)-rhamnose monohydrate (6a, [α]D₂₀ = +8.20°, c = 10, water, 1 h) was purchased from Acros (Geel, Belgium) and used without further purification.
tert-Butyldimethylsilyl 4-O-acetyl-3-azido-2,3,6-trideoxy-β-L-lyxo-hexopyranoside (2c) and tert-butyldimethylsilyl 3-azido-4-O-acetyl-2,3,6-trideoxy-β-L-xylo-hexopyranoside (5c)

Method A. A soln of 9b (3.70 g, ca. 20 mmol) in 1 N HCl (20 mL), AcOH (8 mL) and THF (30 mL) was stirred for 15 h at 20 °C. TLC analysis showed complete cleavage of the acetal group. Sodium azide (2.30 g, ca. 40 mmol) in 1 N HCl (20 mL), AcOH (8 mL) and imidazole (2.72 g, ca. 40 mmol) was added and the reaction mixture was stirred for 10 h. The organic layer was separated and the water phase was extracted with CH2Cl2. The combined organic layer was washed with satd NaHNCO3 and dried with NaSO4. The crude product was purified by column chromatography (1:30 EtOAc–cyclohexane) to give 2c (3.55 g) as a colourless oil, along with 20% unidentified impurity which could not be separated.

tert-Butyldimethylsilyl 3-azido-2,3,6-trideoxy-β-L-lyxo-hexopyranoside (2d).—Deacetylation of 2c (3.40 g, ca. 8 mmol, containing ca. 20% impurity) carried out as described in the synthesis of 3d gave, after column chromatography (1:15 EtOAc–cyclohexane), 2d (2.02 g, 88%) as a colourless solid with mp 69–70 °C (Lit.: 72 °C [17]). [α]D 20 = −3.6° (c = 0.80, CHCl3).

1H NMR (200 MHz, CDCl3): δ = 4.74 (dd, J1,2ax = 8.9, J1,2eq = 2.8, 1 H, 1-H), 3.60 (br s, 1 H, 4-H), 3.46 (dq, J3,4 = 1.0, J5,6 = 6.5, 1 H, 5-H) 3.30 (ddd, J3,4 = 2.7, J3,2ax = 12.5, J3,2eq = 5.1, 1 H, 3-H), 1.99 (ddd, J2ax,2eq = 12.7, 1 H, 2 eq-H), 1.97 (br s, 1 H, 4-OH), 1.84 (ddd, 1 H, 2ax-H), 1.29 (d, 3 H, 6-H), 0.91 (br s, 9 H, SiCMe3).

1O-tert-Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-β-L-lyxo-hexopyranoside (2e).—Catalytic hydrogenation of 2d (1.87 g, 6.5 mmol) carried out as described in the synthesis of 3e gave, after column chromatography (1:5 EtOAc–cyclohexane), 2e (2.11 g, 91%) as a colourless solid with mp 54 °C (Lit.: 55 °C [18]). [α]D 20 = +16.2° (c = 0.92, CHCl3).

1H NMR (300 MHz, CDCl3): δ = 6.82 (br d, JNH,3 = 8, 1 H, NH), 4.77 (dd, J1,2ax = 9.5, J1,2eq = 2.5, 1 H, 1-H), 4.10 (dddd, J3,2ax = 13, J3,2eq = 5.0, J3,4 = 2.3, 1 H, 3-H), 3.62 (qd, J3,4 = 1.0, J5,6 = 6.5, 1 H, 5-H), 3.48 (dd, J4,OH = 10, 1 H, 4-H), 2.12 (d, 1 H, 4-OH), 2.04 (ddd, J2ax,2eq = 13, 1 H, 2eq-H), 1.54 (ddd, 1 H, 2ax-H), 1.28 (d, 3 H, 6-H), 0.88 (s, 9 H, SiCMe3), 0.11 and 0.10 (2 s, 6 H, SiMe2).

1O-tert-Butyldimethylsilyl 4-O-p-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetamido-β-L-lyxo-hexopyranoside (2f).—p-Nitrobenzoylation of 2e (1.97 g, 5.5 mmol) carried out as
described in the synthesis of 3f gave, after column chromatography (1:6 EtOAc–cyclohexane) of the crude product, 2f (2.59 g, 93%) as a colourless solid, mp 73 °C (Lit.: 75 °C [18]). [α]D^25 = −90.1° (c = 0.935, CHCl3). \(^1\)H NMR (300 MHz, CDCl3): \(\delta = 8.32, 8.28\) (A,B, J = 9, 4 H, Ar–H), 6.35 (d, J = 8.14, 1 H, NH), 5.34 (d, J = 5.00, 1 H, 3-H), 4.94 (dd, J = 7.21, 1 H, 2eq-H), 4.37 (m, 1 H, 3-H), 3.85 (qd, J = 5.66, 1 H, J = 5.66, 1 H, 5-H), 2.08 (ddd, J = 12.8, 2ax, 1 H, 1-H), 1.83 (dd, J = 12.8, 1 H, 2ax-H), 1.24 (d, 3 H, 6-H), 0.94 (s, 9 H, SiCMe3), 0.18 and 0.16 (2 s, 6 H, SiMe2).

\(-\)2,3,6-trideoxy-\(\beta\)-L-arabino-hexopyranoside (3b) and \(-\)2,3,6-trideoxy-\(\beta\)-L-ribo-hexopyranose (4b).—A suspension of 7 (17.14 g, 80 mmol) in water (100 mL) was stirred for 2 h at 80 °C. After stirring for 24 h at 20 °C the reaction mixture was poured into a satd aq NaHNCO3 soln and extracted three times with EtOAc (100 mL each time). The organic layer was dried with NaSO4 and evaporated to dryness. The crude mixture of 3b and 4b (16.5 g) was used without further purification and characterisation.

tert-Butyldimethylsilyl \(-\)2,3,6-trideoxy-\(\beta\)-L-arabino-hexopyranoside (3c) and tert-butyldimethylsilyl \(-\)2,3,6-trideoxy-\(\beta\)-L-ribo-hexopyranoside (4c).—tert-Butyldimethylsilyl chloride (13.9 g, 92 mmol) in dry 1,2-dichloroethane (50 mL) was added dropwise to a soln of a crude mixture (16.5 g) and imidazole (10.5 g, 154 mmol) in dry C2H4Cl (200 mL) at 0 °C. After stirring for 24 h, the reaction mixture was washed with water, satd NaHNCO3, and again water. After drying with NaSO4, the crude product was purified by column chromatography (silica gel: 200 g, 30:1 cyclohexane–EtOAc) to give 3c–4c mixture (19.8 g, 60 mmol) and K2CO3 (0.5 g) in dry MeOH (200 mL) was stirred for 15 h. The solvent was removed in vacuo to give a viscous oil (17.4 g) which was purified by column chromatography (silica gel: 400 g, 15:1 cyclohexane–EtOAc). Two fractions were obtained.

From the faster moving fraction, compound 3d (10.2 g, 59%) was obtained as a colourless viscous oil. [α]D^25 = +27.9° (c = 1.87, CHCl3). \(^1\)H NMR (200 MHz, CDCl3): \(\delta = 4.80\) (dd, J = 9.4, 1 H, 1-H), 3.38 (dd, J = 12.5, J = 9.4, 1 H, 5-H), 3.15 (dd, J = 12.5, J = 9.4, 1 H, 3-H), 3.12 (dd, J = 12.5, J = 9.4, 1 H, 5-H), 0.90 (s, 9 H, SiCMe3), 0.13 and 0.12 (2 s, 6 H, SiMe2). Anal. Caled for C24H43N3O3Si: C, 50.34; H, 8.66; N, 14.68.

From the slower moving fraction, compound 4d (4.9 g, 28%) was obtained as a colourless viscous oil. [α]D^25 = −36.6° (c = 0.90, CHCl3). \(^1\)H NMR (200 MHz, CDCl3): \(\delta = 5.00\) (dd, J = 9.4, 1 H, 1-H), 4.07 (dd, J = 9.4, 1 H, 5-H), 3.63 (dq, J = 9.4, J = 6.2, 1 H, 3-H), 3.40 (m, 1 H, 4-H; after D2O-exchange: m → dd), 2.12 (ddd, J = 9.4, J = 6.2, 1 H, 2eq-H), 1.82 (dd, J = 9.4, J = 6.2, 1 H, 2ax-H), 1.29 (d, 3 H, 6-H), 0.91 (s, 9 H, SiCMe3), 0.13 and 0.12 (2 s, 6 H, SiMe2). Anal. Caled for C24H43N3O3Si: C, 50.14; H, 8.77; N, 14.62. Found C, 49.97; H, 9.00; N, 14.55.

1-O-tert-Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-\(\beta\)-L-arabino-hexopyranoside (3e).—Triethylamine (1 mL) and ethyl trifl-
uoroacetate (5.11 g, 36 mmol) was added to a soln of 3d (5.17 g, 18 mmol) in MeOH (50 mL), and the reaction mixture was shaken for 4 h in a hydrogen atmosphere in the presence of Pd–C (10% Pd, 400 mg). After filtering off the catalyst, the solvent was removed in vacuo and the green–brown oil was purified on silica gel (1:5 EtOAc–cyclohexane) to give 3e (5.28 g, 82%) as a colourless solid, mp 140–141 °C (Lit.: 142 °C [18]). 1H NMR (200 MHz, CDCl3): δ = 6.46 (d br, JNH,3 = 7.5 Hz, 1 H, NH), 4.87 (dd, J1,2ax = 8.7, J1,2eq = 2.1 Hz, 1 H, 1-H), 3.96 (m, 1 H, 3-H), 3.37 (qd, J5,4 = 9.1, J5,6 = 6.2 Hz, 1 H, 5-H), 3.17 (t br, J4,3 = 10.0, 1 H, 4-H), 2.47 (s br, 1 H, 4-OH), 2.23 (ddd, J2eq,2ax = 12.7, 1 H, 2eq-H), 1.62 (ddd, J2ax,3 = 12.7, 1 H, 2ax-H), 1.33 (d, 3 H, 6-H3), 0.89 (s, 9 H, SiCMe3), 0.12 and 0.11 (2 s, 6 H, SiMe2). C21H29F3N2O7Si: C, 49.79; H, 5.77; N, 5.53. Anal. Calcd for

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dropwise to a soln of 3e (4.29 g, 12 mmol) and pyridine (5 mL) in CH2Cl2 (40 mL) at 0 °C. After stirring for 12 h at 20 °C, CH2Cl2 (50 mL) and water (50 mL) was added and the mixture was stirred for 30 min. The organic layer was separated, washed with water and dried with Na2SO4. After concentrating in vacuo, the residue was co-evaporated three times with toluene (30 mL each time), and the crude product was purified on silica gel (1:10 EtOAc–cyclohexane) to give 3f (4.45 g, 92%) as a colourless solid, mp 76 °C (Lit. 78 °C [18]). [α]D25 = −23.9° (c = 1.99, CHCl3). 1H NMR (200 MHz, CDCl3): δ = 6.74 (d br, JNH,3 = 7.5, 1 H, NH), 4.87 (dd, J1,2ax = 9.2, J1,2eq = 2.1, 1 H, 1-H), 4.54 (dd, J4,3 = 9.5, 4-H), 4.12 (m, 1 H, 3-H), 3.58 (qd, 1 H, J5,6 = 6.4, 5-H), 2.33 (ddd, 1 H, J2eq,3 = 4.8, J3eq,2eq = 13.0, 2eq-H), 2.08 (s, 3 H, MeCO), 1.62 (ddd, J2ax,3 = 13, 1 H, 2ax-H), 1.24 (d, 3 H, 6-H3), 0.90 (s, 9 H, SiCMe3), 0.12 and 0.11 (2 s, 6 H, SiMe2).

tert-Butyldimethylsilyl 3-azido-2,3,6-trideoxy-4-O-trifluoromethanesulfonyl-β-L-arabinohexopyranoside (3h).—Dry pyridine (3.96 g, 50 mmol) and DMAP (100 mg) was added to 3d (5.75 g, 20 mmol) in dry CH2Cl2 (150 mL). At −40 °C, a soln of trifluoromethanesulfonic acid anhydride (3.4 mL, 6.77 g, 24 mmol) in dry CH2Cl2 (50 mL, containing 1% pyridine) was added dropwise to the reaction mixture. After stirring for 4 h at −20 °C (reaction was monitored by thin layer chromatography (TLC), 1:5 EtOAc–cyclohexane), the reaction mixture was washed twice with 10% aq AcONa, 5% NaHNCO3, and finally with water. The solvent was removed in vacuo, and residue was co-evaporated three times with toluene (30 mL each time) to remove pyridine. The crude product 3h was used directly for the next step (inversion) without further purification. For NMR measurement, a small amount was purified by column chromatography (1:5 EtOAc–cyclohexane) to give 3h as a colourless unstable oil which decomposed within some hours under darkness. 1H NMR (200 MHz, CDCl3): δ = 4.86 (dd, J1,2ax = 9.2, J1,2eq = 2.2, 1 H, 1-H), 4.34 (dd, J3,4 = J4,5 = 9.5, 1 H, 4-H), 3.64 (ddd, J3,2ax = 13, J1,2eq = 5.0, 1 H, 3-H), 3.60 (qd, J5,6 = 6.2, 1 H, 5-H), 2.38 (ddd, J3eq,2eq = 13.0, 1 H, 2eq-H), 1.83 (ddd, 1 H, 2ax-H), 1.38 (d, 3
H, 6-H3), 0.90 (s, 9 H, SiCMe3), 0.13 and 0.12 (2 s, 6 H, SiMe3).

tert-Butyldimethylsilyl 2,3,6-trideoxy-3-trifluoroacetamido-\(\beta\)-L-ribo-hexopyranoside (4e).—Catalytic hydrogenation of 4d (4.00 g, 13.9 mmol) carried out as described in the synthesis of 3e gave, after column chromatography (1:5 EtOAc–cyclohexane), 4e (4.32 g, 87%) as colourless viscous oil. 1H NMR (200 MHz, CDCl3): \(\delta\) = 6.46 (dd br, 1 H, NH), 5.07 (dd, \(J_{1,2ax} = 6.0, J_{1,2eq} = 3.3\), 1 H, 1-H), 4.45 (m, 1 H, 3-H), 3.79 (qd, \(J_{5,4} = J_{5,6} = 6.5, 1 H, 5-H\), 3.65 (s br, 1 H, 4-H), 2.20 (dd, \(J_{2eq,3} = 7.5, J_{2eq,2ax} = 13.5, 1 H, 2eq-H\), 2.10 (s br, 1 H, 4-OH), 1.81 (ddd, \(J_{2ax,3} = 4.1, 1 H, 2ax-H\), 1.39 (d, 3 H, 6-H), 0.89 (s, 9 H, SiMe3), 0.12 and 0.12 (2 s, 6 H, SiMe3). Anal. Calcld for C16H28F3NO5Si: C, 48.11; H, 7.06; N, 3.51.

Found: C, 48.33; H, 7.19; N, 3.64.

tert-Butyldimethylsilyl 3-azido-2,3,6-trideoxy-\(\beta\)-L-xylo-hexopyranoside (5d).

Method A. A soln of 5i (6.00 g, 15.3 mmol) and K2CO3 (0.5 g) in dry MeOH (50 mL) was stirred overnight and solvent was removed in vacuo. Column chromatography (80 g of silica gel, 5:1 cyclohexane–EtOAc) of the crude product yielded 5d (4.00 g, 91%) as a white solid, mp 85–86°C. 1H NMR (200 MHz, CDCl3): \(\delta\) = 4.93 (dd, \(J_{1,2eq} = 4.3 J_{1,2ax} = 7.3, 1 H, 1-H\), 3.98 (dd, \(J_{5,4} = 3.2, J_{2ax,3} = 6.6, 1 H, 3-H\)), 3.91 (dq, \(J_{5,6} = 6.6, J_{5,4} = 1, 1 H, 5-H\)), 3.32 (dd, \(J_{3,1OH} = 1.1 J_{4,1OH} = 4.0, 1 H, 4-H\)), 2.24 (d, 1 H, OH), 1.85 (m, 2 H, 2eq-H, 2ax-H), 1.25 (d, 3 H, 6-H), 0.90 (s, 9 H, Si(C(CH3)3), 0.13, 0.12 (2 s, 2 × 3 H, SiMe3). 13C NMR (75.5 MHz, CDCl3): \(\delta\) = −42.4, −52.1 (Si(CH3)3), 16.59 (C-6), 18.13 ([CH3]C−), 25.75 [−(CH3)3], 32.91 (C-2), 60.47 (C-3), 69.22/68.31(C-4, C-5), 93.12 (C-1).

Method B. Deacetylation of a mixture of 2c–5e was carried out as depicted in the deacetylation of 3c–4c. Thus, when 2.50 g (7.59 mmol) of the mixture was deacetylated in anhyd MeOH (50 mL), using K2CO3 (0.5 g) as the base, a white solid (1.90 g) was obtained. Further separation of the mixture by column chromatography (silica gel: 200 g, 15:1 cyclohexane–EtOAc) afforded two fractions: from the faster moving fraction, compound 2d (0.50 g, 24%) was obtained as a colourless solid with mp 68–70°C. From the slower moving fraction, compound 5d (1.20 g, 56%) was obtained as a colourless solid, mp 85–86°C. Anal. Calcld for C16H28N3O5Si: C, 50.14; H, 8.77. Found: C, 50.11; H, 8.50.

tert-Butyldimethylsilyl 4-O-acetyl-2,3,6-trideoxy-3-trifluoroacetamido-\(\beta\)-L-ribo-hexopyranoside (4g).—Acetylation of 4e (1.79 g, 5 mmol) carried out as described in the synthesis of 3g gave, after column chromatography, 4g (1.77 g, 89%) as a colourless solid with mp 122°C. [x]D25 = +21.4° (c = 0.94, CHCl3). 1H NMR (200 MHz, CDCl3): \(\delta\) = 6.38 (dd br, 1 H, NH), 5.09 (dd, \(J_{1,2ax} = 6.5, J_{1,2eq} = 2.9, 1 H, 1-H\), 4.75 (dd, \(J_{4,3} = 4.0, J_{4,5} = 6.7, 1 H, 3-H\), 4.66 (m, 1 H, 3-H), 3.88 (qd, 1 H, 1-H), 3.78 (dd, 1 H, 5-H), 2.13 (dd, 1 H, 2eq-H, 2.8, \(J_{2ax,2eq} = 13.0, J_{2eq,1H} = 2.08, 1 H, 4-H\)), 1.90 (dd, \(J_{2ax,3} = 4.5, 1 H, 2ax-H\), 1.33 (d, 3 H, 6-H), 0.90 (s, 9 H, SiCMe3), 0.13 and 0.12 (2 s, 6 H, SiMe3). Anal. Calcld for C16H28F3NO5Si: C, 48.11; H, 7.06; N, 3.51.

Found: C, 48.33; H, 7.19; N, 3.64.

tert-Butyldimethylsilyl 3-azido-2,3,6-trideoxy-\(\beta\)-L-xylo-hexopyranoside (5e).—Hydrogenation of 5d (2.40 g, 8.35 mmol) carried out as described in the synthesis of 3e gave, after column chromatography (silica gel: 60 g, 5:1 cyclohexane–EtOAc), 5e (2.65 g, 92%) as a colourless solid, mp 125–126°C. [x]D25 = +16.4° (c = 1.97, CHCl3). 1H NMR (200 MHz, CDCl3): \(\delta\) = 6.26 (br, 1 H, NH), 4.92 (dd, \(J_{1,2eq} = 2.4, J_{1,2ax} = 8.5, 1 H, 1-H\),
4.29 (m, 1 H, 3-H), 3.80 (dq, J5–4 = 1.7, J5–6 = 6.6, 1 H, 5-H), 3.50 (m, J4–3 = 3.6, 1 H, 4-H), 2.30 (d, J = 9.1, 1 H, OH), 2.07 (ddd, J2a,2e = 13.6, J2a–1 = 8.5, J2a,3 = 4.7, 1 H, 2a-H), 1.79 (ddd, J2a,2e = 13.6, J2e–3 = 2.7, J2e,1 = 2.4, 1 H, 2e-H), 1.31 (d, J = 6.6, 3 H, 6-H), 0.90 (s, 9 H, tert-Bu), 0.14 and 0.12 (2 s, 2 × 3 H, –Si–Me).

13C NMR (75.5 MHz, CDCl3): δ = −4.05, −5.17 (–SiMe), 16.73 (C-6), 18.01 [(Me)3SiC]z, 25.70 [–C(Me)3], 33.25 (C-2), 49.92 (C-3), 69.65/68.10 (C-4, C-5), 92.97 (C-1), 115.60 (JC-F = 287.9 Hz, CF3), 156.88 (JC-F = 37.4 Hz, –COCF3). MS (70 eV): m/z (%) = 356.2 ([M+ 7.7%]), 119 (100), 101 (20), 75 (36), 73 (26). Anal. Calcd for C13H26F3NO4Si: C, 47.04; H, 7.34. Found: C, 46.95; H, 7.20.

tert-Butyldimethylsilyl 3-trifluoracetamido-4-O-(p-nitrobenzoyl)-2,3,6-trideoxy-L-xylo-hexopyranoside (5f).—p-Nitrobenzoylation of 5e (1.55 g, 4.34 mmol) carried out as described in the synthesis of 3f gave, after column chromatography (silica gel: 60 g, 20:1 cyclohexane–EtOAc) to give 5f (7.0 g, 88%) as a colourless viscous oil. [α]D20 = −60.4° (c = 0.37, CHCl3). 1H NMR (200 MHz, CDCl3): δ = 8.09 (m, 2 H) and 7.65–7.42 (m, 3 H, phenyl–H), 5.06 (dd, J1,2ax = 3.9, J1,2ax = 7.3, 1 H, 1-H), 4.81 (dd, J4,5 = 3.2, J4,5 = 1.5, 1 H, 4-H), 4.10 (m, 2 H, 3–, 5–H), 1.93 (m, 2 H, 2–H), 1.25 (d, J6,5 = 6.5, 3 H, 6–H). 0.94 (s, 9 H, SiC(CH3)3), 0.17, 0.16 (2 s, 2 × 3 H, SiMe2).

3,4-Di-O-acetyl-1,5-anhydro-2,6-dideoxy-L-arabino-hex-1-enol (di-O-acetyl-L-(+)-rhamninal) (7).—Rhamnose (6a) (100 g, 0.55 mol) was added to a mixture of Ac2O (340 mL) and 70% perchloric acid (2 mL) in a 1-L three-necked flask in such a way that the reaction temperature was maintained between 30 and 40°C. After stirring for 2 h, phosphorus tribromide (70 mL) was added dropwise below 10°C. Finally water (33 mL) was added dropwise below 15°C, and the reaction mixture was then stirred for another 2 h at 20°C.

Sodium acetate (226 g), water (700 mL) and AcOH (295 mL) were added to a 4-L three-necked round bottom flask equipped with mechanical stirrer, thermometer and dropping funnel. The mixture was cooled to −10°C using MeOH–dry-ice bath. Zinc powder (200 g) and finally aq CuSO4 soln (16.5 g CuSO4 × 5 H2O in 65 mL water) were added.

After disappearance of the blue colour and development of hydrogen, the crude bromo-aceto–rhamnose soln prepared above was added dropwise to this mixture and the reaction temperature was maintained between
16 and 03 45/Pcar2121y.

The reaction mixture was stirred for another 5 min. Unreacted zinc powder and Cu were filtered off, and the filtrate was extracted five times with CH₂Cl₂ (300 mL each time). The combined organic layer was washed with ice–water, satd NaHNCO₃, again ice–water, and dried with NaSO₄. The solvent was removed in vacuo and the crude product was vacuum-distilled at 78–79 °C/0.9 Torr to give 7 (100 g, 85%) as a colourless liquid (Lit.: 68–69 °C/0.06 Torr [9]). ¹H NMR (200 MHz, CDCl₃): δ = 6.43 (dd, 1 H, J₁,₂ = 6.1, 1-H), 5.34 (m, 1 H, 2-H), 5.03 (dd, 1 H, J₁,₅₄ = 8.1, J₃,₄ = 6.1, 4-H), 4.78 (dd, 1 H, J₃,₄ = 6.1, J₂,₃ = 3.0, 3-H), 4.11 (dq, 1 H, J₄,₅ = 6.1, J₅,₆ = 6.6, 5-H), 2.09, 2.05 (2 s, 6 H, 2 AcO), 1.32 (d, 3 H, J₅,₆ = 6.6, 6-H).

**Method A.** Compound 8b (8.00 g, ca. 10 mmol) was dissolved in dry MeOH (30 mL) and anhyd K₂CO₃ (0.1 g) was added. After stirring overnight, the solvent was removed in vacuo. The residue was dissolved in dry pyridine (10 mL) and mesyl chloride (2.06 g, ca. 18 mmol) was added dropwise at 0 °C.

After stirring for 15 h at 20 °C, water (30 mL) was added and the mixture was extracted with CH₂Cl₂. The crude product was dried with NaSO₄, and redissolved in DMF (50 mL). Caesium benzoate (3.81 g) was added and the suspension was stirred for 5 h at 80 °C. At 20 °C, water (50 mL) was then added and the reaction mixture was extracted four times with diethyl ether (50 mL of each time). The organic phase was washed with water, dried with Na₂SO₄, and the crude product was purified by column chromatography to give 9a (0.95 g, 39%) as a colourless oil. [α]D²⁵ = +214.0° (c = 0.86, CHCl₃).

**Method B (Mitsunobu reaction).** To a soln of 8c (9.6 g, 66.6 mmol), benzoic acid (17.0 g, 0.14 mol) and triphenylphosphine (36.5 g, 0.14 mol) in anhyd THF (100 mL), DEAD (22.0 g, ca. 0.13 mol) dissolved in THF (30 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 15 h and the solvent was removed in vacuo, 100 mL of ether was added to the residue and the insoluble part was filtered off. After removal of the solvent, the crude product was purified by column chromatography (200 g of silica gel, 10:1 cyclohexane–EtOAc) to give 9a (15.00 g, 90%) as a colourless viscous liquid. ¹H NMR (200 MHz, CDCl₃): δ = 8.09 (m, 2 H, Ar–H), 7.61–7.39 (m, 3 H, Ar–H), 6.21 (dd, J₃,₄ = 5, J₃,₂ = 10, 1 H, 3-H), 6.06 (dd, J₂,₁ = 1, 1 H, 2-H), 5.15 (dd, J₄,₅ = 5.2, 1 H, 4-H), 4.98 (d, 1 H, 1-H), 4.33 (dq, J₅,₆ = 6.5, 1 H, 5-H), 3.47 (s 3 H, OMe), 1.32 (d, 3 H, 6-H). β-9a: δ = 8.09 (m, 2 H, Ar–H), 7.61–7.39 (m, 3 H, Ar–H), 6.18 (dd, J₃,₁ = 1.5, J₃,₄ = 4.8, J₃,₂ = 10, 1 H, 3-H), 6.06 (dd, J₂,₁ = 2.8, J₅,₆ = 6.5, 1 H, 5-H), 3.53 (s 3 H, OMe), 1.34 (d, 3 H, 6-H).

**Methyl 4-O-acetyl-2,3,6-trideoxy-α,β-L-threohex-2-enopyranoside (9b)**

Deacetylation reaction. A mixture of anhyd K₂CO₃ (0.1 g) and 8b (6.50 g, ca. 35 mmol) in dry MeOH (30 mL) was stirred for 15 h at 20 °C and solvent was removed in vacuo. The resulting yellowish oil was purified on silica gel (column, 10:1 cyclohexane–EtOAc) to give 8c (4.70 g) as a colourless oily mixture of both anomers.

**Mitsunobu reaction.**—Diethyl azodicarboxylate (11.40 g, ca. 65 mmol) in dry THF (15 mL) was added dropwise to the soln of 8c
(4.7 g, 65 mmol) obtained above, AcOH (4.0 g, ca. 65 mmol) and triphenylphosphine (17.10 g, 65 mmol) in dry THF (100 mL) at 0 °C. The reaction mixture was stirred 15 h and solvent was removed in vacuo, diethyl ether (50 mL) was added to the residue and the insoluble part was filtered off. After removal of solvent, the crude product was purified by column chromatography (200 g silica, 10:1 cyclohexane–EtOAc) to give 9b (4.85 g, 85%) as a colourless viscous liquid.

7-O-(2,3,6-Trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamidomethyl-L-arabinob-hexopyranosyl)-\(\varepsilon\)-iso-rhodycinone (7-O-acosaminyL-\(\varepsilon\)-iso-rhodycinone) (10).—Me\(_3\)Si– triflate (1.50 g, 6.72 mmol) was added dropwise at −35 °C to a suspension of 1b (1.80 g, 4.05 mmol), 2f (2.60 g, 5.13 mmol) and powdered molecular sieve (2 A\(_\text{g}\), 4.5 g) in 10:1 CH\(_2\)Cl\(_2\)–acetone (210 mL). After stirring for 6 h at this temperature, the reaction was quenched by addition of triethylamine (2.8 mL). The molecular sieve was filtered off, and the filtrate was washed twice with water (100 mL each time). After drying with NaSO\(_4\), the crude product was purified by column chromatography (170 g of silica gel, 200:10:1 chloroform–EtOAc–for-

Cell culture.—Human tumor cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO\(_2\)) in monolayer cultures in RPMI 1640 medium with phenol red (Life Technologies, Karlsruhe, Germany) supplemented with 10% of fetal calf serum. Cells were trypsinised and maintained weekly. 

Cytotoxicity assay.—A modified propidium iodide assay was used to examine the antiproliferative activity of the studied compounds [19]. Briefly, cells were harvested from exponential phase cultures growing in RPMI 1640 medium supplemented with 10% of fetal calf serum by trypsination, counted and plated in 96 well flat-bottomed microtitre plates (100 μl cell suspension, 1 × 10\(^5\) and 5 × 10\(^4\) cells/mL). After a 24 h recovery, to allow cells to resume exponential growth, 50 μl culture medium (six control wells per plate) or culture medium containing the test drug were added to the wells. Each drug concentration was plated in triplicate. After 3–7 days of incubation, depending on cell doubling time, culture medium was replaced by fresh medium containing pro-
pidium iodide (6 μg/mL). Microplates were then kept at −18 °C for 24 h, resulting in a total cell kill. After thawing of the plates, fluorescence was measured using a Millipore Cytofluor 2350-microplate reader (excitation 530 nm, emission 620 nm) in order to quantify the total cell number. The assay included untreated and positive controls (Doxorubicin).

Growth inhibition was expressed as treated/control × 100 (T/C%). IC₅₀ and IC₇₀ values were determined by plotting compound concentration versus cell number. Mean IC₅₀ and IC₇₀ values were calculated according to the formula:

\[
\frac{X}{n}
\]

With \( X \) = specific tumor cell line and \( n \) = total number of cell lines studied. If IC₅₀ or IC₇₀ could not be determined within the examined dose range, the lowest or highest concentration studied was used for the calculation.

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References


