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Note

Stereo- and regioselective glycosylation of 4-deoxy-ε-rhodomycinone

Filip S. Ekholm [†], Lucas Lagerquist [†], Reko Leino [*]

Laboratory of Organic Chemistry, Åbo Akademi University, FI-20500 Åbo, Finland

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ABSTRACT

A method for the glycosylation of anthracyclines featuring benzoylated imidate donors has been developed and utilized in the synthesis of glycosylated 4-deoxy-ε-rhodomycinone derivatives. Due to its high efficiency, regioselectivity, stereoselectivity, and operational simplicity, the method should prove valuable to researchers working with glycosylation of tetracyclic compounds.

During the 1950s, a search for compounds with potential anticancer activity began by isolation of substances from soil-living microbes.[1] This resulted in the isolation of the first group of anthracyclines, the actinomycins.[2] Since then, a rapid expansion of this field has taken place.[3] Anthracyclines are compounds consisting of a sugar residue attached to a tetracyclic aglycone core. Anthracyclines, such as doxorubicin and daunorubicin (Fig. 1), possess many biologically and clinically important properties and have found widespread use in the treatment of leukemia, breast carcinomas, and other solid tumors.[4] While being one of the most potent groups of antineoplastic agents, the use of anthracyclines has thus far been limited due to their undesired side effects, such as myelosuppression, gastrointestinal disorders, and cumulative cardiotoxicity.[5] In order to reduce the number of side-effects associated with anthracyclines, a vast number of anthracyclines have been prepared, thereby resulting also in advances in glycosylation methodologies and isolation processes.[3,6] By these routes, both naturally occurring and synthetically modified anthracycline derivatives have been produced and evaluated, generating increased knowledge about the biological mechanism of their action.[7] Although several glycosylation methodologies have been developed, many of them suffer from poor selectivity, efficiency, and long reaction times.[8]

Herein, we report a simple, efficient, and highly selective glycosylation procedure for the glycosylation of anthracyclines, as exemplified by the synthesis of a limited set of glycosylated 4-deoxy-ε-rhodomycinone derivatives.

Rhodomycinone is an intermediate on the biosynthetic pathway leading to doxorubicin.[9] For evaluation of glycosylation methodologies, a closely related tetracyclic compound, 4-deoxy-ε-rhodomycinone, was utilized in the present study.[10] Since the 4-deoxy-ε-rhodomycinone tetracyclic core closely resembles the structure of other biologically active anthracyclines, such as daunorubicin, it was chosen as a suitable model substrate for the evaluation of glycosylation methodologies. Due to the large number of glycosylation methodologies developed previously, we initially screened several widely utilized carbohydrate donors, such as halide-, thio- and glycal-donors, in the glycosylation reaction.[6a,11] While biochemical synthesis and isolation processes have contributed significantly to the identification and production of novel anthracycline derivatives, such methods were not screened within the scope of the present work.[12]

Glycosyl halides were the first carbohydrate donors utilized in the synthesis of glycosides.[13] As a result, these donors have also been applied frequently to the synthesis of anthracyclines.[14] The most utilized procedure for glycosylation using bromo- and chloro-donors is the Koenigs–Knorr reaction.[15] Although widely used, the need for activating agents such as mercuric salts or silver triflate, which are both toxic and expensive, can be considered as considerable drawbacks.[16] Even more importantly, the Koenigs–Knorr reaction usually proceeds with moderate efficiency (yields ranging between 40% and 60%).[8a,14a,17] In addition, the reaction times reported in the literature have been surprisingly long.[8a,13a] Regardless of the drawbacks, bromo and chloro sugars were evaluated as glycosyl donors also in the present study. Moderate yields (30–55%) were obtained when acetobromo glucose and galactose were applied as donors. In addition to the moderate yields, poor stereoselectivity was observed. When the acetyl groups were

* Corresponding author. Tel.: +358 2 2154132; fax: +358 2 2154866.
E-mail address: reko.leino@abo.fi (R. Leino).
† These authors contributed equally to this work.

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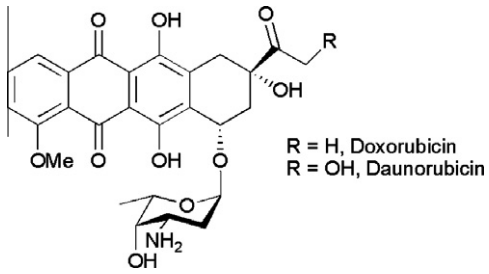


Figure 1. Structures of two well known anthracyclines doxorubicin and daunorubicin.

replaced by benzoyl groups, the stereoselectivity was, however, significantly improved simultaneously increasing the overall yield of the glycosylation (up to 79%). Although these yields were already in the desired range, the need for toxic and expensive promoters led us to search for a more convenient glycosylation strategy.

Thioglycosides and glycals represent other classes of commonly used carbohydrate donors.[18] Both classes have been utilized for the glycosylation of anthracyclines. In many of the reported studies, these donors have resulted in moderate regioselectivity, stereoselectivity, and efficiency.[8b,19] Both classes were also screened in the present study, however, with limited success. Therefore, we then progressed by evaluating another class of glycosyl donors, the imidate donors.

Surprisingly, imidate donors have not received similar attention as the other donors in anthracycline glycosylations. Although not used as frequently with anthracyclines, imidate-donors have found wide use in the glycosylation of biomolecules and have in several studies been found to be optimal in terms of efficiency and operational simplicity. Since the benzoylated bromo-donors proved to be more efficient than the corresponding acetylated donors, we turned our attention to benzoylated imidates. The benzoylated imidate donors have in previous studies been found to be superior in comparison to the acetylated imidate donors in terms of efficiency.[20] We envisioned that the benzoylated imidate donors would be more suited also in the present study due to the larger size of the benzoyl groups in comparison to acetyl groups, which potentially could enhance the regioselective outcome of the glycosylation. In addition, ester-protective groups at C-2 increase the overall stereoselectivity of the glycosidation by neighboring group participation.[21] In an initial experiment, TMSOTf and $BF_3{\cdot}OEt_2$ were screened as promoters. The weaker Lewis acid TMSOTf proved to be superior and was thus utilized as a promoter in the remaining experiments. Following the standard activation protocol, by the inverse glycosylation approach, using only 0.2 equiv of TMSOTf as a promoter and 1.1 equiv of donor we were able to isolate 97% of the 7-O-glucosylated 4-deoxy-ε-rhodomycinone. Encouraged by these initial results, we further prepared the imidate donors **2**, **5**, **8,** and **11** and evaluated these in the glycosylation reactions. All reactions proceeded with conversions ranging form 93–98% (based on NMR). The products were simply filtered after the reaction and utilized as such in the final deprotection step (Table 1).

The regioselectivities of the products were easily determined from the HMBC correlation between H-7 and C'-1. The stereoselectivities of the reactions were determined based on the H-1–H-2 coupling constants and compared to the literature values.[22] Deprotection under Zemplén conditions proceeded smoothly to give the desired compounds in high yields.[23] It was noticed that upon addition of sodium methoxide, the solution turned purple and upon neutralization back to red, thus providing a simple way of monitoring the conditions in the final deprotection. Owing to the amphiphilic character of the deprotected molecules, the compounds could be purified by column chromatography thus providing a simple method for removing the methyl benzoate formed as a side product in the final deprotection. To the best of our knowledge, this is the first time that benzoylated imidate donors have been applied to the glycosylation of anthracyclines and based on the synthesis of the limited anthracycline library, presented in this paper, they may find further use in the glycosylations of anthracyclines. Furthermore, the methodology utilizes only 1.1 equiv of donor and 0.2 equiv of promoter as compared to other procedures where 2–3 equiv of both donor and promoter are applied.

To conclude, we have shown that benzoylated imidate donors are suitable alternatives, in comparison with other carbohydrate donors, for glycosylation of anthracyclines. The method described herein, utilized in the synthesis of a limited set of glycosylated anthracyclines, should prove valuable due to its high efficiency, regioselectivity, stereoselectivity, and operational simplicity. In future work, we plan to assess the biological activity of the synthesized molecules and extend the methodology to investigate also deoxy-sugar based donors.

1. Experimental

1.1. General methods

Reaction solvents were dried and distilled prior to use when necessary. All reactions containing moisture- or air-sensitive reagents were carried out under an argon atmosphere.

The NMR spectra were recorded with Bruker Avance spectrometer operating at 600.13 MHz (^{1}H: 600.13 MHz, ^{13}C: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C unless otherwise mentioned. All products were fully characterized by utilization of the following 1D-techniques; ^{1}H, 1D-TOCSY, and ^{13}C in combination with the following 2D-techniques; DQF-COSY, NOESY, HSQC, and HMBC by using pulse sequences provided by the manufacturer.

Chemical shifts are expressed on the δ scale (in ppm) using TMS (tetramethylsilane), residual chloroform, acetone, H_2O, or MeOH as internal standards. Coupling constants are given in Hertz and provided only once when first encountered. Coupling patterns are given as s, singlet, d, doublet, t, triplet, etc. The computational analysis of the ^{1}H NMR of all compounds was achieved by utilization of the PERCH NMR software with starting values and spectral parameters obtained from the various NMR techniques used.[24]

HRMS were recorded using Bruker Micro Q-TOF with ESI (electrospray ionization) operated in positive mode. TLC was performed on aluminum sheets precoated with Silica Gel 60 F_{254} (Merck). Flash chromatography was carried out on Silica Gel 60 (0.040–0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:10 H_2SO_4/MeOH and heating.

1.2. Syntheses

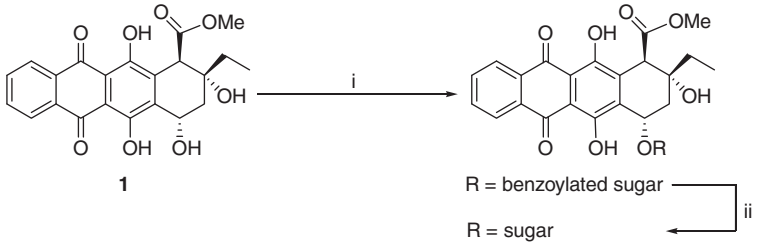
1.2.1. General procedure for glycosylation

To a solution containing 4-deoxy-ε-rhodomycinone (1 equiv) and pre-activated 4 Å MS in dry CH_2Cl_2 (1.6 ml/0.1 mmol substrate) was added TMSOTf (0.2 equiv) at −40 °C. The reaction mixture was stirred for 10 min and the corresponding donor (1.1 equiv) dissolved in dry CH_2Cl_2 (1.6 ml/0.1 mmol substrate) was added dropwise to the solution. The resulting mixture was stirred at −30 °C for 1.5–2.0 h, brought to rt, diluted with dry CH_2Cl_2 (20 ml), filtered, and concentrated. The crude product was used as such in the deprotection step.

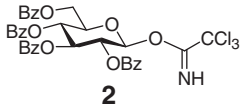
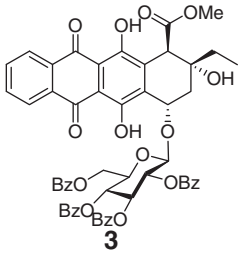
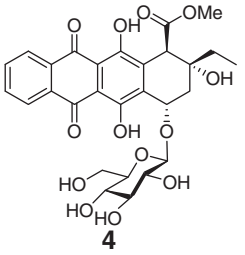
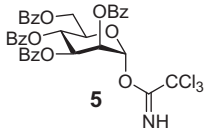
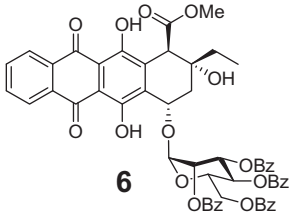
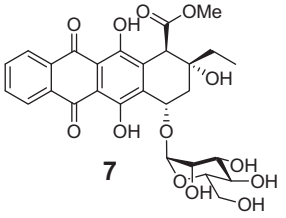
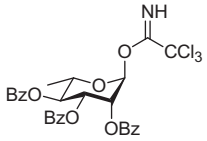
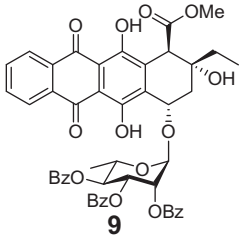
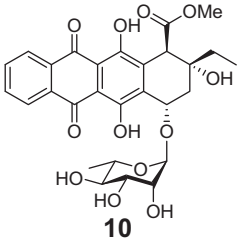
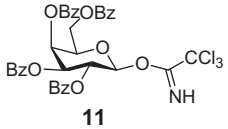
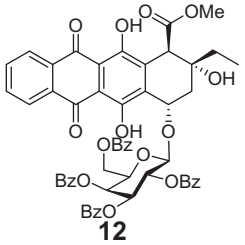
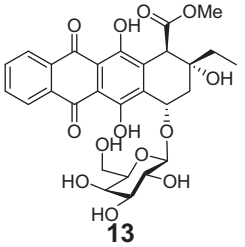
1.2.2. General procedure for deprotection

To a solution containing the protected anthracycline glycoside (1 equiv) in a mixture MeOH/THF (2:1, 6 ml /0.1 mmol substrate)

Table 1
Summary of the glycosylations and deprotections performed, reaction conditions and yields



i) 1) TMSOTf, CH_2Cl_2, −40° 2) glycosyl donor, −40°→0°, 2 h; ii) NaOMe, MeOH:THF, 2–16 h.

Glycosyl donor	Glycosylation product	Glycosylation yield[a]	Deprotection product	Deprotection yield[a]
 **2**	 **3**	96%	 **4**	97%
 **5**	 **6**	Not determined[b]	 **7**	86% (over two steps)
 **8**	 **9**	Not determined[b]	 **10**	90% (over two steps)
 **11**	 **12**	Not determined[b]	 **13**	92% (over two steps)

[a] Isolated yields.
[b] Reaction mixture was filtered and subjected to deprotection.

was added NaOMe (3 equiv) and upon addition the color of the mixture turned purple. The reaction mixture was stirred between 3 and 15 h, neutralized with DOWEX 50 H^+-form (color turned back to red), filtered and concentrated. The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ 1:10→MeOH/CH_2Cl_2 1:3) to give the deprotected anthracycline.

1.2.3. 7-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glucopyranosyl)-4-deoxy-ε-rhodomycinone (3)

Synthesized from **1** (29 mg, 0.07 mmol) and **2** (57 mg, 0.077 mmol) according to the general procedure for glycosylation providing **3** as a red solid (67 mg, 96%). R_f = 0.96 (in $MeOH/CH_2Cl_2$ 1:10); ^1H NMR (600.13 MHz, $CDCl_3$): δ 13.41 (s, 1H, 6-O*H*), 13.18 (s,

1H, 11-O*H*), 8.29–6.90 (m, 24 H, arom. *H*), 6.02 (dd, 1H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 10.0 Hz, H-3′), 5.72 (dd, 1H, $J_{4',5'}$ = 10.0 Hz, H-4′), 5.56 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.45 (dd, 1H, H-2′), 5.34 (dd, 1H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 4.2 Hz, H-7), 4.76 (dd, 1H, $J_{6'a,5'}$ = 3.1, $J_{6'a,6'b}$ = −12.1 Hz, H-6′a), 4.58 (dd, 1H, $J_{6'b,5'}$ = 5.0 Hz, H-6′b), 4.32 (d, 1H, $J_{10,8a}$ = −1.2 Hz, H-10), 4.30 (ddd, 1H, H-5′), 3.67 (s, 3 H, 10-COOC*H*$_3$), 2.55 (ddd, 1H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.20 (dd, 1H, H-8b), 1.73 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −13.9 Hz, H-13a), 1.45 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.10 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 186.7 (C-5), 186.2 (C-12), 171.7 (10-*C*OOCH$_3$), 166.3 (6′-*O*COPh), 165.8 (3′-*O*COPh), 165.3 (4′-*O*COPh), 165.2 (2′-*O*COPh), 156.3 (C-6), 156.1 (C-11), 135.2–128.1 (arom. *C*), 111.7 (C-11a), 111.2 (C-5a), 103.1 (C-1′), 72.5 (C-5′), 72.4 (C-3′), 72.1 (C-2′), 71.8 (C-7), 70.8 (C-9), 69.8 (C-4′), 63.0 (C-6′), 52.4 (10-COO*C*H$_3$), 51.4 (C-10), 34.7 (C-8), 32.8 (C-13), 6.8 (C-14).

HRMS: calcd for $C_{56}H_{46}O_{17}Na_1$ [M+Na]$^+$ 1013.2633; found 1013.2668.

1.2.4. 7-*O*-(β-D-Glucopyranosyl)-4-deoxy-ε-rhodomycinone (4)

Synthesized from **3** (91 mg, 0.09 mmol) according to the general procedure for deprotection providing **4** as a red solid (51 mg, 97%). R_f = 0.14 (in MeOH/CH_2Cl_2 1:10). ^{1}H NMR (600.13 MHz, CD_3OD + $CDCl_3$): δ 8.20–7.75 (m, 4 H, arom. *H*), 5.29 (dd, 1H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.5 Hz, H-7), 4.91 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.24 (d, 1H, $J_{10,8a}$ = −1.2 Hz, H-10), 3.93 (dd, 1H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = −11.8 Hz, H-6′a), 3.79 (dd, 1H, $J_{6'b,5'}$ = 5.1 Hz, H-6′b), 3.70 (s, 3 H, 10-COOC*H*$_3$), 3.47 (dd, 1H, $J_{3',4'}$ = 8.9, $J_{3',2'}$ = 9.3 Hz, H-3′), 3.43 (ddd, 1H, $J_{5',4'}$ = 9.8 Hz, H-5′), 3.39 (dd, 1H, H-4′), 3.22 (dd, 1H, H-2′), 2.66 (ddd, 1H, $J_{8a,8b}$ = −15.1 Hz, H-8a), 2.22 (dd, 1H, H-8b), 1.71 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.57 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.15 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD + $CDCl_3$): δ 188.1 (C-5), 187.9 (C-12), 172.8 (10-*C*OOCH$_3$), 157.8 (C-6), 156.8 (C-11), 137.6–127.9 (arom. *C*), 112.9 (C-11a), 112.6 (C-5a), 106.2 (C-1′), 78.1 (C-5′), 77.7 (C-3′), 75.5 (C-2′), 72.3 (C-7), 71.9 (C-9), 71.3 (C-4′), 62.5 (C-6′), 52.8 (10-COO*C*H$_3$), 52.0 (C-10), 36.2 (C-8), 34.1 (C-13), 7.2 (C-14).

HRMS: calcd for $C_{28}H_{30}O_{13}Na_1$ [M+Na]$^+$ 597.1584; found 597.1588.

1.2.5. 7-*O*-(2′,3′,4′,6′-Tetra-*O*-benzoyl-α-D-mannopyranosyl)-4-deoxy-ε-rhodomycinone (6)

Synthesized from **1** (31 mg, 0.074 mmol) and **5** (61 mg, 0.081 mmol) according to the general procedure for glycosylation providing **6** as an orange solid (containing 10% of hydrolyzed donor) (107 mg). R_f = 0.58 (in hexane/EtOAc 1:1) . ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 13.80 (s, 1H, 6-O*H*), 13.40 (s, 1H, 11-O*H*), 8.40–7.21 (m, 24 H, arom. *H*), 6.23 (dd, 1H, $J_{4',3'}$ = 10.1, $J_{4',5'}$ = 10.2 Hz, H-4′), 5.82 (dd, 1H, $J_{3',2'}$ = 3.2 Hz, H-3′), 5.64 (d, 1H, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.64 (dd, 1H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 3.9 Hz, H-7), 5.61 (dd, 1H, H-2′), 4.96 (ddd, 1H, $J_{5',6'a}$ = 2.5, $J_{5',6'b}$ = 3.6 Hz, H-5′), 4.83 (dd, 1H, $J_{6'a,6'b}$ = −12.2 Hz, H-6′a), 4.58 (dd, 1H, H-6′b), 4.48 (d, 1H, $J_{10,8a}$ = −1.1 Hz, H-10), 3.74 (s, 3 H, 10-COOC*H*$_3$), 2.53 (ddd, 1H, $J_{8a,8b}$ = −15.4 Hz, H-8a), 2.20 (dd, 1H, H-8b), 1.86 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −13.9 Hz, H-13a), 1.57 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.21 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 187.1 (C-5), 187.0 (C-12), 171.7 (10-*C*OOCH$_3$), 166.4 (6′-*O*COPh), 165.7 (2′-*O*COPh), 165.6 (4′-*O*COPh, 3′-*O*COPh), 157.0 (C-6), 156.3 (C-11), 136.4–129.1 (arom. *C*), 112.5 (C-11a), 111.9 (C-5a), 95.1 (C-1′), 71.1 (C-2′), 71.0 (C-9), 70.2 (C-5′), 70.0 (C-3′), 67.7 (C-7), 66.6 (C-4′), 62.9 (C-6′), 52.6 (10-COO*C*H$_3$), 52.1 (C-10), 32.7 (C-13), 29.8 (C-8), 6.9 (C-14).

HRMS: calcd for $C_{56}H_{46}O_{17}Na_1$ [M+Na]$^+$ 1013.2633; found 1013.2626.

1.2.6. 7-*O*-(α-D-Mannopyranosyl)-4-deoxy-ε-rhodomycinone (7)

Synthesized from **6** (107 mg (containing impurities), 0.1 mmol) according to the general procedure for deprotection providing **7** as a red solid (37 mg, 86% (over two steps)). R_f = 0.16 (in MeOH/CH_2Cl_2 1:10). ^{1}H NMR (600.13 MHz, CD_3OD + $CDCl_3$): δ 8.37–7.85 (m, 4 H, arom. *H*), 5.53 (dd, 1H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 3.9 Hz, H-7), 5.28 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.34 (d, 1H, $J_{10,8a}$ = −1.1 Hz, H-10), 4.14 (ddd, 1H, $J_{5',6'a}$ = 2.9, $J_{5',6'b}$ = 3.3, $J_{5',4'}$ = 10.0 Hz, H-5′), 3.94 (dd, 1H, $J_{6'a,6'b}$ = −12.1 Hz, H-6′a), 3.93 (dd, 1H, H-6′b), 3.82 (dd, 1H, $J_{4',3'}$ = 9.5, Hz, H-4′), 3.81 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.72 (s, 3 H, 10-COOC*H*$_3$), 3.68 (dd, 1H, H-3′), 2.48 (ddd, 1H, $J_{8a,8b}$ = −15.3 Hz, H-8a), 2.07 (dd, 1H, H-8b), 1.79 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.1 Hz, H-13a), 1.54 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.15 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD + $CDCl_3$): δ 186.7 (C-5), 186.4 (C-12), 171.3 (10-*C*OOCH$_3$), 156.1 (C-6), 155.3 (C-11), 135.1–126.6 (arom. *C*), 111.8 (C-11a), 111.3 (C-5a), 97.0 (C-1′), 73.4 (C-5′), 70.8 (C-3′), 70.7 (C-2′), 70.5 (C-9), 66.3 (C-4′), 65.3 (C-7), 60.9 (C-6′), 51.9 (10-COO*C*H$_3$), 51.6 (C-10), 32.2 (C-13), 29.1 (C-8), 6.0 (C-14).

HRMS: calcd for $C_{28}H_{30}O_{13}Na_1$ [M+Na]$^+$ 597.1584; found 597.1588.

1.2.7. 7-*O*-(2′,3′,4′,6′-Tetra-*O*-benzoyl-α-L-rhamnopyranosyl)-4-deoxy-ε-rhodomycinone (9)

Synthesized from **1** (25 mg, 0.06 mmol) and **8** (41 mg, 0.067 mmol) according to the general procedure for glycosylation providing **9** as an orange solid (containing 10% of hydrolyzed donor) (68 mg). R_f = 0.74 (in hexane/EtOAc 1:1) . ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 13.53 (s, 1H, 6-O*H*), 13.37 (s, 1H, 11-O*H*), 8.36–7.17 (m, 19 H, arom. *H*), 5.82 (dd, 1H, $J_{2',1'}$ = 1.9, $J_{2',3'}$ = 3.4 Hz, H-2′), 5.73 (dd, 1H, $J_{4',5'}$ = 9.8, $J_{4',3'}$ = 10.1 Hz, H-4′), 5.65 (d, 1H, H-1′), 5.64 (dd, 1H, H-3′), 5.40 (dd, 1H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.4 Hz, H-7), 4.45 (dq, 1H, $J_{5',6'}$ = 6.2 Hz, H-5′), 4.40 (d, 1H, $J_{10,8a}$ = −1.2 Hz, H-10), 3.75 (s, 3 H, 10-COOC*H*$_3$), 2.50 (ddd, 1H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.36 (dd, 1H, H-8b), 1.92 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.54 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.45 (d, 1H, H-6′), 1.20 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 187.1 (C-12), 186.7 (C-5), 171.5 (10-*C*OOCH$_3$), 165.9 (4′-*O*COPh), 165.4 (2′-*O*COPh, 3′-*O*COPh), 156.8 (C-6), 156.1 (C-11), 134.6–127.2 (arom. *C*), 112.3 (C-11a), 111.9 (C-5a), 101.5 (C-1′), 72.8 (C-7), 71.6 (C-4′), 71.1 (C-9), 70.6 (C-2′), 70.0 (C-3′), 68.1 (C-5′), 52.6 (10-COO*C*H$_3$), 52.0 (C-10), 33.8 (C-8), 32.4 (C-13), 17.7 (C-6′), 6.9 (C-14).

HRMS: calcd for $C_{49}H_{42}O_{15}Na_1$ [M+Na]$^+$ 893.2421; found 893.2400.

1.2.8. 7-*O*-(L-Rhamnopyranosyl)-4-deoxy-ε-rhodomycinone (10)

Synthesized from **9** (65 mg (containing impurities), 0.074 mmol) according to the general procedure for deprotection providing **10** as a red solid (30 mg, 90% over two steps). R_f = 0.45 (in EtOAc). ^{1}H NMR (600.13 MHz, CD_3OD + $CDCl_3$): δ 8.30–7.82 (m, 4 H, arom. *H*), 5.38 (d, 1H, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.25 (dd, 1H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.5 Hz, H-7), 4.26 (d, 1H, $J_{10,8a}$ = −1.3 Hz, H-10), 3.95 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.88 (dq, 1H, $J_{5',6'}$ = 6.2, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.73 (s, 3 H, 10-COOC*H*$_3$), 3.55 (dd, 1H, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.48 (dd, 1H, H-4′), 2.40 (ddd, 1H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.16 (dd, 1H, H-8b), 1.84 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.50 (dq, 1H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.38 (d, 1H, H-6′), 1.15 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD + $CDCl_3$): δ 186.5 (C-5), 186.4 (C-12), 171.3 (10-*C*OOCH$_3$), 156.2 (C-6), 155.4 (C-11), 134.9–126.6 (arom. *C*), 111.4 (C-11a), 111.2 (C-5a), 104.1 (C-1′), 72.2 (C-4′), 71.4 (C-7), 70.8 (C-3′), 70.6 (C-9), 70.3 (C-2′), 69.3 (C-5′), 51.9 (10-COO*C*H$_3$), 51.4 (C-10), 33.6 (C-8), 32.0 (C-13), 16.7 (C-6′), 6.1 (C-14).

HRMS: calcd for $C_{28}H_{30}O_{12}Na_1$ $[M+Na]^+$ 581.1635; found 581.1624.

1.2.9. 7-*O*-(2′,3′,4′,6′-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-4-deoxy-ε-rhodomycinone (12)

Synthesized from **1** (41 mg, 0.10 mmol) and **11** (82 mg, 0.11 mmol) according to the general procedure for glycosylation providing **12** as a red solid (containing 10% hydrolyzed donor) (136 mg). R_f = 0.94 (in MeOH/CH_2Cl_2 1:10); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 13.42 (s, 1H, 6-O*H*), 13.20 (s, 1H, 11-O*H*), 8.32–6.90 (m, 24 H, arom. *H*), 6.06 (dd, 1H, $J_{4',5'}$ = 1.4, $J_{4',3'}$ = 3.5 Hz, H-4′), 5.77 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, H-3′), 5.68 (dd, 1H, $J_{2',1'}$ = 8.0 Hz, H-2′), 5.55 (d, 1H, H-1′), 5.39 (dd, 1H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 4.2 Hz, H-7), 4.78 (dd, 1H, $J_{6'a,5'}$ = 6.5, $J_{6'a,6'b}$ = −11.2 Hz, H-6′a), 4.50 (ddd, 1H, $J_{5',6'b}$ = 6.6 Hz, H-5′), 4.49 (dd, 1H, H-6′b), 4.37 (d, $J_{10,8a}$ = 1.2 Hz, 1H, H-10), 3.69 (s, 3 H, 10-COOC*H*$_3$), 2.61 (ddd, 1H, $J_{8a,8b}$ = −14.9 Hz, H-8a), 2.26 (dd, 1H, H-8b), 1.75 (dq, 1H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.55 (dq, 1H, $J_{13b,14}$ = 7.4 Hz, H-13b), 1.20 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 186.5 (C-5), 186.0 (C-12), 171.5 (10-*C*OOCH$_3$), 166.0 (6′-*C*OPh), 165.6 (4′-*C*OPh), 165.4 (3′-*C*OPh), 165.1 (2′-*C*OPh), 156.1 (C-6), 155.9 (C-11), 135.2–126.6 (arom. *C*), 111.5 (C-11a), 111.0 (C-5a), 103.3 (C-1′), 71.7 (C-7), 71.6 (C-5′), 71.0 (C-9), 70.7 (C-3′), 69.8 (C-2′), 68.1 (C-4′), 62.0 (C-6′), 52.3 (10-COO*C*H$_3$), 51.0 (C-10), 34.8 (C-8), 32.9 (C-13), 6.7 (C-14).

HRMS: calcd for 1013.2633; found 1013.2564.

1.2.10. 7-*O*-(β-D-Galactopyranosyl)-4-deoxy-ε-rhodomycinone (13)

Synthesized from **12** (136 mg, 0.1 mmol) according to the general procedure for deprotection providing **13** as a red solid (54 mg, 92% yield over two steps). R_f = 0.17 (in MeOH/CH_2Cl_2 1:10). ^{1}H NMR (600.13 MHz, CD_3OD + $CDCl_3$): δ 8.33–7.86 (m, 4 H, arom. *H*), 5.30 (dd, 1H, $J_{7,8a}$ = 1.9, $J_{7,8b}$ = 4.3 Hz, H-7), 4.83 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1′), 4.28 (s, 1H, H-10), 3.94 (dd, 1H, $J_{4',5'}$ = 1.4, $J_{4',3'}$ = 3.2 Hz, H-4′), 3.85 (dd, 1H, $J_{6'a\text{-}5'}$ = 5.8, $J_{6'a\text{–}6'b}$ = −11.7 Hz, H-6′a), 3.84 (dd, 1H, $J_{6'b\text{-}5'}$ = 6.3 Hz, H-6′b), 3.72 (s, 3 H, 10-COOC*H*$_3$), 3.67 (ddd, 1H, H-5′), 3.62 (dd, 1H, $J_{3',2'}$ = 9.5 Hz, H-3′), 3.56 (dd, 1H, H-2′), 2.69 (ddd, 1H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.21 (dd, 1H, H-8b), 1.75 (dq, 1H, $J_{13a,14}$ = 7.1, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.53 (dq, 1H, $J_{13b,14}$ = 7.6 Hz, H-13b), 1.14 (dd, 1H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD + $CDCl_3$): δ 186.5 (C-5, C-12), 171.3 (10-*C*OOCH$_3$), 156.1 (C-6), 155.3 (C-11), 134.6–126.5 (arom. *C*), 111.4 (C-11a), 111.2 (C-5a), 105.1 (C-1′), 74.8 (C-5′), 72.8 (C-3′), 71.4 (C-7), 71.1 (C-2′), 70.1 (C-9), 68.2 (C-4′), 60.6 (C-6′), 51.7 (10-COO*C*H$_3$), 50.3 (C-10), 34.0 (C-8), 32.5 (C-13), 5.9 (C-14).

HRMS: calcd for $C_{28}H_{30}O_{13}Na_1$ $[M+Na]^+$ 597.1584; found 597.1577.

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Supplementary data

Supplementary data (spectral data of all final compounds are provided) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.01.028.

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