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Semisynthetic ε-isorhodomycins: their synthesis using glycals and their structure–activity relationship

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ABSTRACT

Syntheses and structure–activity relationships of 7-*O*-(3-amino-2,3,6-trideoxy-α-L-*lyxo*- (**18**), -L-*arabino*- (**20**) and -L-*ribo*-hexopyranosyl)-ε-isorhodomycins (**25**) and their 3′-dimethylamino derivatives **22**, **23** and **26** are described. Condensation (trimethylsilyl triflate, molecular sieves 4 Å, 10:1 dichloromethane–acetone, −15°) of ε-isorhodomycinone (ε-isoRMN, **6**) with 1,5-anhydro-4-*O*-*p*-nitrobenzoyl-3-trifluoroacetamido-L-*lyxo*- (**5**) -L-*arabino*- (**9**) or -L-*ribo*-hex-1-enitols (**10**) afforded mainly the 7-*O*-α-glycosyl-ε-isoRMNs **7**, **11**, and **12**. Similar glycosylation of **6** with 1,5-anhydro-3-azido-4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-L-*arabino*-hex-1-enitol (**15**) yielded α-glycoside **16**. Removal (M NaOH) of the *p*-nitrobenzoyl and trifluoroacetyl groups from **7**, **11**, and **12** gave the 7-*O*-(3-amino-2,3,6-trideoxy-α-L-hexopyranosyl)-ε-isoRMNs **18**, **20**, and **25**. Reductive alkylation (CH_2O, $NaCNBH_3$) of these products afforded the 3′-*N*,*N*-dimethyl analogues **22**, **23**, and **26**. The cytotoxic effect (IC_{50}) of the semisynthetic ε-isorhodomycins was tested *in vitro* in leukemia cell line L1210.

INTRODUCTION

In recent years reports[1,2] have focused on anthracyclines of the doxorubicin–daunorubicin group and on the microbial β-rhodomycins, but ε-(iso)rhodomycins have received little attention. This may be attributed to the only moderate cytostatic efficacy of the ε-(iso)rhodomycins thus far isolated or prepared semisynthetically, e.g. **1** (ref. 3), **2** (ref. 3), **3** (ref. 4), or **4** (ref. 5).

The most important step in the synthesis of anthracyclines is glycosylation. The glycosyl donors of the 3-amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose (daunosamine) type used in the condensation step are typical 4-*O*-*p*-nitrobenzoyl or 4-*O*-trifluoroacetyl and 3-*N*-trifluoroacetyl derivatives. The amino sugar is activated for coupling either *via* the glycosyl halide[2,6], glycal[2,7,8], or *p*-nitrobenzoate[9].

Few reports exist on the synthesis of the glycosidic linkage in ε-(iso)rhodomycinone glycosides. El Khadem *et al.*[5] prepared ε-rhodomycins by allowing ε-RMN to react with a large excess of the *O*-acetylated sugar halide in the presence of mercuric salts. The synthesis of 7-*O*-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-ε-RMN was described by Smith *et al.*[4], who showed that condensation of ε-RMN with 4 equiv. of 4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetylamino-α-L-*lyxo*-hexopyranosyl chloride stereoselectively results in the α-glycoside. On the other hand, the aglycons are able

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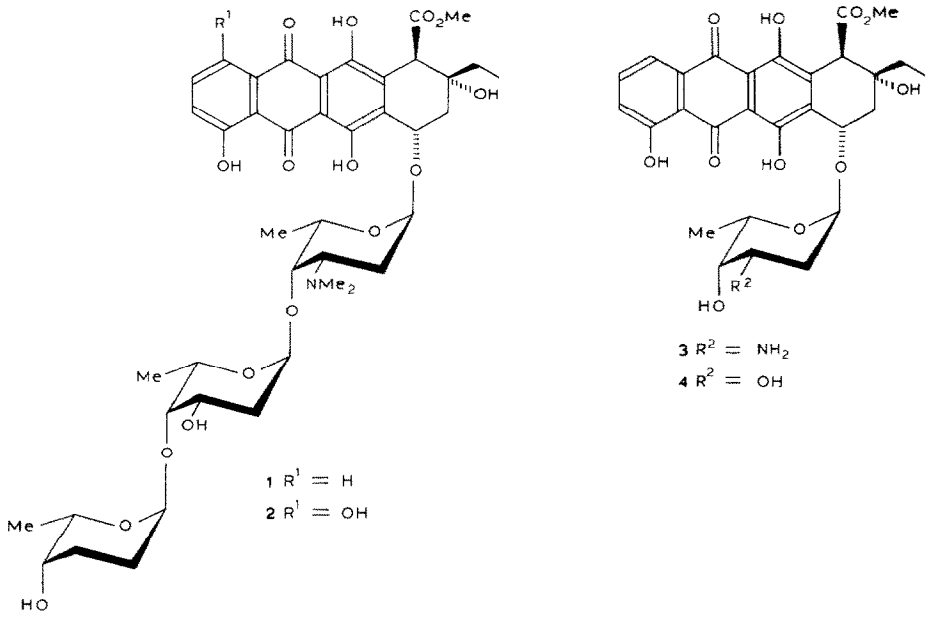


Scheme 1

to be glycosylated by microorganisms. The mutant KE 303 derived from *Streptomyces galilaeus* produces cinerulosyl-deoxyfucosyl-rhodosaminyl-rhodomycinones when fed with aglycon[3]. We report a new method for the glycosylation of ε-isoRMN using 1,5-anhydro-L-*lyxo*-, -L-*arabino*-, or -L-*ribo*-hex-1-enitols and discuss the structure–activity relationship of semisynthetic ε-isorhodomycins[10,11].

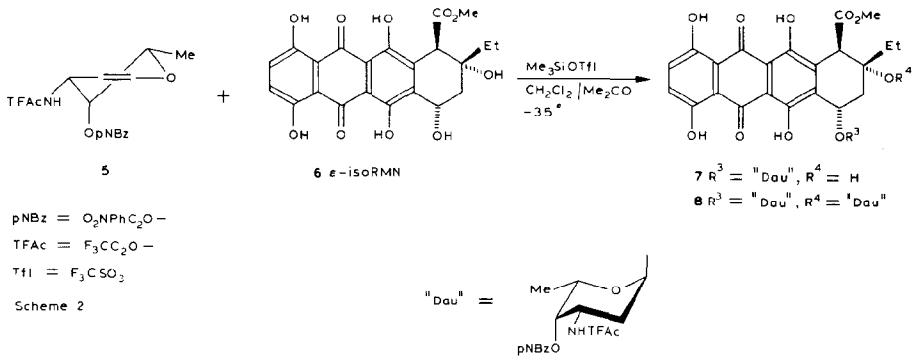
RESULTS AND DISCUSSION

Thus far, 1,5-anhydro-4-*O*-*p*-nitrobenzoyl-3-trifluoroacetylamino-L-*lyxo*- (**5**) -L-*arabino*- (**9**) or -L-*ribo*-hex-1-enitols (**10**) have been used for glycosylation of anthracyclinones, yielding mainly α-glycosides in the presence of *p*-toluenesulfonic acid (TsOH) as promoter, in yields[7,8] of 40–60%. Anomeric stereospecificity ($\alpha:\beta$ = 100:0) was only observed[8] in the reaction of **10** with daunomycinone.

We investigated the use of Me_3Si triflate as promoter instead of TsOH. Glycosylation (Me_3Si triflate, molecular sieves 4 Å, 10:1 dichloromethane–acetone, −35°) of ε-isoRMN[12]* **6** with **5** afforded the desired α-glycoside **7** (54%) but, because of the high reactivity of the glycal **5**, a considerable amount of glycoside **7** was converted into 7,9-bis(*O*-α-glycosyl)-ε-isoRMN **8**. At a lower temperature (−50°) condensation occurred more slowly, but it was not possible to suppress the formation of **8**.

Glycosylation of ε-isoRMN **6** with **9** or **10** under the conditions (Me_3Si triflate, molecular sieves 4 Å, 10:1 dichloromethane–acetone) described for the preparation of **7**,

* We are indebted to HIL Bombay for gifts of ε-isorhodomycinone.

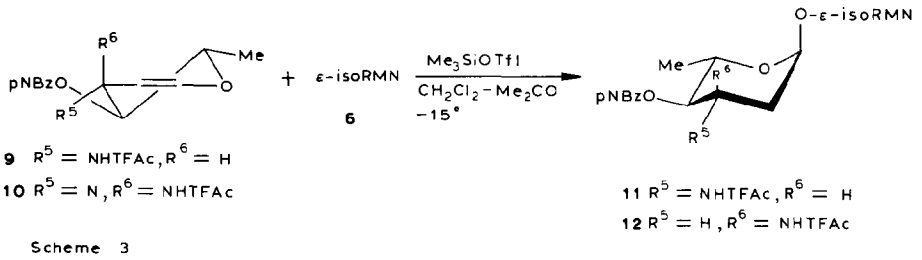


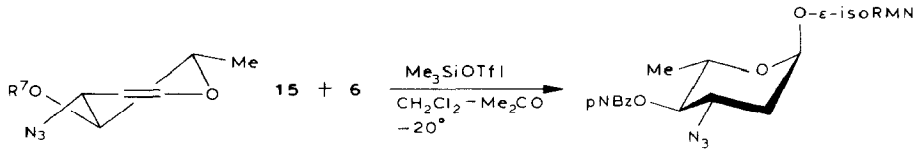
but at −15°, afforded 7-*O*-α-glycosyl-ε-isoRMNs **11** and **12** in 76% yield. In t.l.c. the 7,9-bis-*O*-glycosylated by-products were found in trace amounts only.

Furthermore we applied our procedure for the synthesis of 3-azido-2,3,6-trideoxy-α-L-*arabino*-hexopyranosides[13] using 1,5-anhydro-3-azido-4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-L-*arabino*-hex-1-enitol (**15**) as the glycosyl donor. Compound **15** was prepared from its 4-*O*-benzoyl-analogue[8] **13** in a 2-stage reaction sequence — *O*-debenzoylation of **13** to **14** by treatment with sodium methoxide, followed by esterification of **14** with *p*-nitrobenzoyl chloride in the presence of pyridine–dichloromethane. Condensation (Me_3Si triflate, molecular sieves 4 Å, 10:1 dichloromethane–acetone, −15°) of **6** with **15** afforded α-glycoside **16** (76%) in full anomeric purity[13].

The structures of all of the following glycosyl-ε-isoRMNs was confirmed beyond doubt by means of ^{1}H-n.m.r. spectroscopy and ^{1}H,^{1}H-COSY experiments.

As reported[10], 7-*O*-(4-*O*-*p*-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetamido-α-L-*lyxo*-hexopyranosyl)-ε-isoRMN (**7**) may be deprotected either partially to provide **17** by removing the *p*-nitrobenzoyl group with 0.1M NaOH or completely (M NaOH) to provide 7-*O*-(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-ε-isoRMN (**18**). Likewise, deprotection of **11** and **12** with M NaOH in 2:1 chloroform–methanol gave the 7-*O*-[3-amino-2,3,6-trideoxy-α-L-*arabino*- (**20**) and -α-L-*ribo*-hexopyranosyl]-ε-isoRMNs (**25**); the *p*-nitrobenzoyl group were removed first, followed by the trifluoroacetyl group. The 3′-*N*-trifluoroacetyl intermediates **19** and **24** may be obtained in good yield by treatment with 0.1M NaOH. The 3′-azidorhodomycin **16** was deprotected first by removal (0.1M NaOH) of the *p*-nitrobenzoyl group followed by treatment of the azido intermediate **21** with sodium borohydride–ammonium chloride in dichloromethane–





13 R^7 = Bz
14 R^7 = H
15 R^7 = pNBz

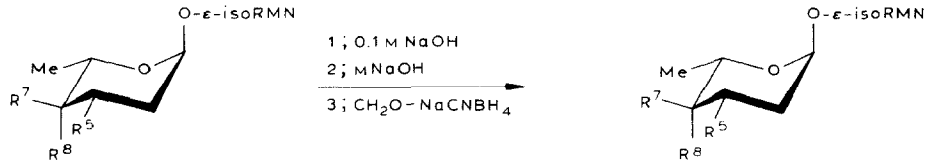
16

Scheme 4

ethanol–water. The reaction product was identical to the already described compound **20**. ^{1}H-N.m.r. examination of the deprotected rhodomycins **18**, **20**, and **25** in 10:1 $CDCl_3$–MeOD indicated a common $^1C_4$-conformation for their sugars moieties and a half-chair form for their aglycon A-rings.

ε-Isorhodomycins **18**, **20**, and **25** were converted into the 3′-dimethylamino derivatives **22** (ref. 10), **23**, and **26** under conventional conditions of reductive alkylation (CH_2O, $NaCNBH_3$). ^{1}H,^{1}H-COSY experiments were used to assign the sugar and rhodomycinone A-ring protons within the ^{1}H-n.m.r. spectra ($CDCl_3$) of **22**, **23**, and **26**. As expected, the 3-dimethylamino-L-*lyxo*- and -L-*arabino*-hexopyranosyl moieties in **22** or **23** are in the $^1C_4$-conformation. In contrast, the existence of a boat conformation $B_{O,3}$ of the 3-dimethylamino-L-*ribo*-hexopyranosyl moiety in **26** was indicated by ^{1}H-n.m.r. studies, in which the $J_{2'a,3'}$ (11.0 Hz) and $J_{4',5'}$ (6.8 Hz) values are found to be consistent, H-2′*a*,3′ being *trans*-diaxial and H-4′,5′ being equatorial–axial, respectively.

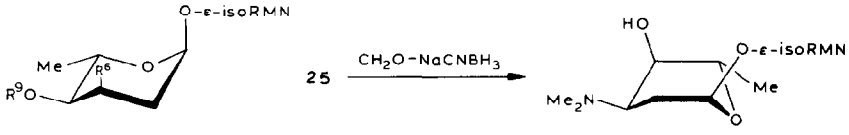
The cytostatic activity of the semisynthetic ε-isorhodomycins was tested on L1210 mouse leukemia cells in a clonogenic assay[15]. This method is used to detect the effect of the test substances on growth behavior of the cells over 1 h or 7 days (about 14 consecutive generations with a cell cycle lasting 10–12 h). The results are stated as percentages of the surviving colonies in the treated groups versus the untreated groups. The cytotoxicity (IC_{50}, μg/mL) for continuous and one-h incubation were determined from the dose–effect curve (Table I). For comparative purposes, Table I shows anti-



7 R^5 = NHTFAc, R^7 = H, R^8 = OpNBz
11 R^5 = NHTFAc, R^7 = OpNBz, R^8 = H
16 R^5 = N, R^7 = OpNBz, R^8 = H

17 R^5 = NHTFAc, R^7 = H, R^8 = OH
18 R^5 = NH_2, R^7 = H, R^8 = OH
19 R^5 = NHTFAc, R^7 = OH, R^8 = H
20 R^5 = NH_2, R^7 = OH, R^8 = H
21 R^5 = N_3, R^7 = OH, R^8 = H
22 R^5 = NMe_2, R^7 = H, R^8 = OH
23 R^5 = NMe_2, R^7 = OH, R^8 = H

Scheme 5



12 R^6 = NHTFAc, R^9 = pNBz
24 R^6 = NHTFAc, R^9 = H
25 R^6 = NH_2, R^9 = H

26

Scheme 6

tumor assay data for doxorubicin (**26**), 3′-*N*,*N*-dimethyldoxorubicin[16] (**27**), and 7-*O*-(3-methylamino-2,3,6-trideoxy-*α*-L-*lyxo*-hexopyranosyl)-ε-isoRMN[10] (**30**).

The resulting data lead us to the following conclusions concerning the structure–activity relationship:

the cytotoxicity of ε-isorhodomycins is increased by alkylation of the 3′-amino group in the sequence $NH_2 < NHMe < NMe_2$;

the equatorial disposition of the 3′-amino group is essential for the development of the cytotoxic effect;

the low cytostatic activity of the ε-isorhodomycins might be attributed to the high lipophilicity of these compounds.

Compared to adriamycin **27**, none of the semisynthetic isorhodomycins exhibited a greater or similar activity in the *in vitro* test for cytotoxicity, except for 7-*O*-*α*-rhodosaminyl-ε-isoRMN (**22**). Further modification of this structure, especially the alkylation of the 3′-amino group in 7-*O*-*α*-L-daunosaminyl-ε-isoRMN (**18**), could well improve the antitumor efficacy of ε-isorhodomycins.

TABLE I

Cytotoxicity (IC_{50}) of semisynthetic ε-isorhodomycins in L1210 (*in vitro*) screening system with incubation times of 1 h and 7 days

Compound	**3**	**16**	**18**	**20**	**22**	**23**	**25**	**26**	**27**	**28**	**29**	**30**
IC_{50}(μg/mL) 7 days	>1	>1	0.6	>1	0.02	0.12	>1	>1	0.02	0.01	0.5	0.28
IC_{50}(μg/mL) 1 h	>1	>1	>1	>1	1.9	>1	>1	>1	0.04	>1	>1	>1

EXPERIMENTAL

General methods. — Reactions were carried out at ambient temperature unless otherwise stated. Solutions were evaporated under diminished pressure at <40° (bath). Organic solutions were washed with 0.1M KH_2PO_4 or 0.1M sodium citrate adjusted to the appropriate pH value using 0.1M NaOH or 0.1M HCl. Melting points, determined on a Büchi apparatus, are uncorrected. ^{1}H-N.m.r. spectra were recorded with Bruker AC-200, AC-300, and AM-400 or Jeol GX400 spectrometers, on solutions in $CDCl_3$ (internal Me_4Si) unless stated otherwise. The ^{1}H resonances were assigned by ^{1}H,^{1}H-

COSY experiments, using the standard pulse-sequences of the Bruker Aspect-3000 software. Specific optical rotations were determined with a Perkin–Elmer 241 polarimeter equipped with 10-cm cuvettes, for solutions in $CHCl_3$ at 24°, unless noted otherwise. Reactions were monitored by t.l.c. on Silica Gel 60 F 254 (Merck) with detection by u.v. light or by charring with H_2SO_4. Preparative chromatography was performed on Kieselgel 60 (Merck, 0.015–0.040 mm). The glycosylations were performed under argon or nitrogen.

General procedures. — (a) Glycosylation of ε-isoRMN **6** *with glycals* **9**, **10**, and **15**. To a stirred mixture of ε-isoRMN **6** (1.0 mmol), glycal (2.0–2.5 mmol) and powdered molecular sieves 4 Å (880 mg) in 10:1 CH_2Cl_2–acetone (250 mL), was added trimethylsilyl trifluoromethanesulfonate ($Me_3SiOTfl$, 890 mg, 4.0 mmol) at −15°. After 2 h stirring, CH_2Cl_2 (50 mL) and Et_3N (1 mL) were added, the mixture was filtered, washed with 0.1M citrate buffer (pH 4.5, 50 mL), 0.1M phosphate buffer (pH 7.5, 50 mL), and water (50 mL x 2), dried (Na_2SO_4), and evaporated. Column chromatography (eluent as described for t.l.c.) of the residue on silica gel (~70 g) gave the glycoside.

(b) Deprotection of p-nitrobenzoyl group in ε-isorhodomycins **7**, **11**, **12**, and **16**. To a stirred mixture of *p*-nitrobenzoate (0.855 mmol) in 2:1 $CHCl_3$–MeOH (10 mL) was added aqueous 0.1M NaOH (10 mL) at room temperature. After 1 h, the mixture was made neutral with aq. 0.1M HCl (10 mL) and evaporated. The residue was suspended in 5:1 $CHCl_3$–MeOH (80 mL), washed with 0.1M phosphate buffer (pH 7.5, 30 mL × 3) and saturated NaCl (20 mL). The organic layer was dried (Na_2SO_4) and evaporated. Column chromatography (eluent as described for t.l.c.) of the residue on a column of silica gel (~60 g) gave the 4′-*O*-deprotected product.

(c) Deprotection of the trifluoroacetyl group in ε-isorhodomycins **7**, **11**, and **12**. To a stirred mixture of protected ε-isorhodomycin (0.855 mmol) in 2:1 $CHCl_3$–MeOH (15 mL) was added aq. M NaOH (15 mL). After 30 min the mixture was diluted with $CHCl_3$ (50 mL), made neutral with aq. M HCl (15 mL) and evaporated. The residue was dissolved in 1:1 CH_2Cl_2–BuOH (70 mL) and the solution stirred with Na_2SO_4 (2.0 g) for 30 min. The inorganic salts were filtered off and washed with 1:1 CH_2Cl_2–BuOH. The combined filtrate and washings were evaporated. Column chromatography (eluent as described for t.l.c.) of the residue on a column of silica gel (~200 g) gave deprotected product.

(d) Reductive methylation of the amino group in ε-isorhodomycins **18**, **20**, and **25**. To a solution of ε-isorhodomycin (0.435 mmol) in aq. CH_2O (37% CH_2O, 0.65 mL) and MeOH (25 mL) was added $NaCNBH_3$ (165 mg). The mixture was stirred for 4 h at room temperature and then evaporated. The residue was dissolved in 1:1 CH_2Cl_2–BuOH (20 mL) and stirred with Na_2SO_4 (2 g) for 30 min. The inorganic salts were filtered off and washed, and the combined filtrate and washings evaporated. Column chromatography (eluent as described for t.l.c.) of the residue on a column of silica gel (~60 g) gave the 3′-*N*,*N*-dimethylated product.

*7-O-(4-O-p-Nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetylamido-α-*L-lyxo-*hexopyranosyl)-ε-isorhodomycinone* (**7**) *and 7,9-bis-*O-*(4-*O-*p-nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetylamido-α-*L-lyxo-*hexopyranosyl)-ε-isorhodomycinone* (**8**). — To a stirred

mixture of ε-isoRMN **6** (2.0 g, 4.50 mmol), glycal **5** (1.82 g, 4.86 mmol) and powdered molecular sieves 4 Å (4.0 g) in 10:1 CH_2Cl_2–acetone (400 mL) was added $Me_3SiOTfl$ (0.52 g, 2.34 mmol) at −35°. After ~2 h stirring at −35°, Et_3N (2 mL) was added, and the mixture was stirred for a further 10 min, filtered and the solid washed with CH_2Cl_2 (150 mL). The filtrates were combined, washed with 0.1M citrate buffer (pH 4.5, 100 mL × 2), 0.1M phosphate buffer (pH 7.5, 100 mL) and water (150 mL), dried (Na_2SO_4) and evaporated. The residue was crystallized from diethyl ether–light petroleum to give a mixture of **7** and **8**. Column chromatography (200:10:1 $CHCl_3$–EtOAc–HCO_2H) of the crystallized product on silica gel (160 g) gave **7** (1.98 g, 54%) and **8** (1.23 g, 23%).

Compound **7** had m.p. 195–197°, $[\alpha]_D$ +135° (*c* 0.01); lit.[10] m.p. 195–197°, $[\alpha]_D$ +135°.

Compound **8** had m.p. 218–223°, $[\alpha]_D$ +46.4° (*c* 0.0065); ^{1}H-n.m.r. (400 MHz): δ 13.14, 12.95, 12.39 and 12.31 (4s, 4 H, HO-1,4,6,11), 8.36–8.24 (m, 8 H, nitroarom. H), 7.40 (d, 1 H, $J_{3'',NH}$ 5.1 Hz, NH-3''), 7.32 (s, 2 H, H-2,3), 6.97 (d, 1 H, $J_{3',NH}$ 7.3 Hz, NH-3'), 5.64 (d, 1 H, $J_{1'',2''a}$ 3.1 Hz, H-1''), 5.47 (d, 1 H, $J_{1',2'a}$ 2.4 Hz, H-1'), 5.42 (bs, 1 H, H-4'), 5.24 (bs, 1 H, H-4''), 5.07 (d, 1 H, $J_{7,8b}$ 5.5 Hz, H-7), 4.84 (d, 1 H, $J_{8a,10}$ 1.1 Hz, H-10), 4.70 (q, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 4.60 (m, 1 H, H-3''), 4.57 (m, 1 H, H-3'), 3.79 (q, 1 H, $J_{5'',6''}$ 6.5 Hz, H-5''), 3.73 (s, 3 H, CO_2Me), 2.58 (d, 1 H, $J_{A,B}$ 15.0 Hz, H-8-A), 2.30 (m, 1 H, H-2''e), 2.25 (m, 1 H, H-13a), 2.21 (dd, 1 H, $J_{7,8b}$ 5.5 Hz, H-8-B), 2.19 (m, 1 H, H-2'e), 2.07 (m, 1 H, H-2'*a*), 2.04 (m, 1 H, H-2''*a*), 1.35 (m, 1 H, $J_{13,14}$ 6.4 and, $J_{A,B}$ 14.8 Hz, H-13B), 1.35 (d, 3 H, $J_{5',6'}$ 6.6 Hz, H-6'), 1.07 (t, 3 H, $J_{13,14}$ 6.4 Hz, H-14), and 0.62 (d, 3 H, $J_{5'',6''}$ 6.4 Hz, H-6'').

Anal. Calc. for $C_{52}H_{46}F_6N_4O_{22}$ (1192.95): C, 52.36; H, 3.89; N, 4.70. Found: C, 52.42; H, 3.87; N, 4.52.

*7-O-(4-O-p-Nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetylamido-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**11**). — The reaction of ε-isoRMN **6** (440 mg, 0.99 mmol) and glycal **11** (9.35 mg, 2.5 mmol) in the presence of $Me_3SiOTfl$ (890 mg, 4.0 mmol) at −15° as described in general procedure (*a*) gave **11** (621 mg, 76%), m.p. 279–281°, $[\alpha]_D$ +1070° (*c* 0.1), t.l.c. in 15:1 PhMe–acetone. ^{1}H-N.m.r. (400 MHz): δ 13.02, 12.83, 12.29, and 12.29 (4s, 4 H, HO-1,4,6,11), 8.31–8.18 (m, 4 H, nitroarom. H), 7.28 (s, 2 H, H-2,3), 6.63 (d, 1 H, $J_{3',NH}$ 8.1 Hz, N-H), 5.51 (d, 1 H, $J_{1',2'a}$ 3.8 Hz, H-1'), 5.24 (dd, 1 H, $J_{7,8a}$ 1, $J_{7,8b}$ 4.4 Hz, H-7), 4.91 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, $J_{4',5'}$ 10.0 Hz, H-4'), 4.42 (m, 1 H, H-5'), 4.37 (m, 1 H, H-3'), 4.32 (s, 1 H, H-10), 4.09 (s, 1 H, HO-9), 3.74 (s, 3 H, CO_2Me), 2.47 (dd, 1 H, $J_{2'a,2'e}$ 12.8, $J_{2'e,3'}$ 4.5 Hz, H-2'e), 2.42 (d, 1 H, $J_{A,B}$ 15.1 Hz, H-8a), 2.30 (dd, 1 H, H-8b), 1.94 (ddd, $J_{2'a,3'}$ 12.8 Hz, H-2'a), 1.91 (m, 1 H, $J_{13,14}$ 7.3 Hz, $J_{A,B}$ 15.0 Hz, H-13b), 1.33 (d, 3 H, $J_{5',6'}$ 6.1 Hz, H-6'), and 1.81 (t, 3 H, H-14).

Anal. Calc. for $C_{37}H_{33}F_3N_2O_{16}$ (818.67): C, 54.28; H, 4.06; N, 3.42. Found: C, 54.31; H, 4.06; N, 3.40.

*7-O-(4-O-p-Nitrobenzoyl-2,3,6-trideoxy-3-trifluoroacetylamino-α-*L-ribo-*hexopyranosyl)-ε-isorhodomycinone* (**12**). — The reaction of ε-isoRMN **6** (1.0 g, 2.25 mmol) and glycal **10** (1.68 g, 4.48 mmol) in the presence of $Me_3SiOTfl$ (0.45 g, 2.02 mmol) at −15° according to general procedure (*a*) yielded **12** (1.40 g, 76%), m.p. 162–164°, $[\alpha]_D$ +1093° (*c* 0.01), t.l.c. in 95:0.5:0.2:0.1 $CHCl_3$–acetone–water–Et_3N. ^{1}H-N.m.r. (300 MHz): δ 13.03, 12.86, 12.33 and 12.30 (4s, 4 H, HO-1,4,6,11), 8.26–8.05 (m, 4 H,

nitroarom. H), 8.16 (d, 1 H, $J_{3',NH}$ 8.4 Hz, N-H), 7.29 (s, 2 H, H-2,3), 5.47 (d, 1 H, $J_{1',2'a}$ 2.6 Hz, H-1'), 5.11 (d, 1 H, $J_{7,8b}$ 4.0 Hz, H-7), 4.95 (dd, 1 H, $J_{3',4'}$ 4.0, $J_{4',5'}$ 10.4 Hz, H-4'), 4.77 (m, 1 H, H-3'), 4.47 (qd, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 4.21 (s, 1 H, H-10), 3.72 (s, 3 H, CO_2Me), 2.42 (d, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.22 (dd, 1 H, H-8b), 2.22 (d, 1 H, $J_{2'a,2'e}$ 14.0 Hz, H-2'*e*), 2.07 (dd, 1 H, H-2'*a*), 1.86 (m, 1 H, $J_{13,14}$ 7.5 Hz, $J_{A,B}$ 15.0 Hz, H-13a), 1.62 (m, 1 H, H-13b), 1.32 (d, 3 H, H-6'), and 1.30 (d, 3 H, H-14).

Anal. Calc. for $C_{37}H_{33}F_3N_2O_{16}$ (818.67): C, 54.28; H, 4.06; N, 3.42. Found: C, 54.32; H, 4.05; N, 3.37.

*1,5-Anhydro-3-azido-4-*O*-p-nitrobenzoyl-2,3,6-trideoxy-*L-arabino-*hex-1-enitol* (**15**). — A mixture of 1,5-anhydro-3-azido-4-*O*-benzoyl-L-*arabino*- and -*ribo*-hex-1-enitol[8] (3.5 g, containing about 2.0 g of **13**) was dissolved in MeOH (100 mL) and methanolic M NaOMe was added until pH 11 was achieved. The mixture was stirred for 1 h and then made neutral with Dowex Wx8 on pH 7.5. The resin was filtered off, washed and the combined filtrate and washings were evaporated. Column chromatography (1:1 diethyl ether–hexane) of the residue on silica gel (250 g) gave 1,5-anhydro-3-azido-2,3,6-trideoxy-L-*arabino*-hex-1-enitol (**14**, 1.2 g). To a solution of **14** (1.2 g) in 1:1 CH_2Cl_2–pyridine (50 mL) was added *p*-nitrobenzoyl chloride (2 g) at 0°. The mixture was stirred for 1 h at 0° to room temperature and evaporated. The residue was dissolved in CH_2Cl_2 (60 mL), washed with 0.1M phosphate buffer (pH 8, 50 mL × 3), dried ($MgSO_4$) and evaporated. Toluene (50 mL × 3) was evaporated from the residue. Column chromatography (5:5:0.5 CH_2Cl_2–light petroleum–EtOAc) of the crude product on silica gel (80 g) gave **15** (2.04 g, 87%), m.p. 98–100°, $[\alpha]_D$ +214.7° (*c* 1.0); *v* max 2100 cm^{-1} (N_3); ^{13}C-n.m.r. (90 MHz): δ 163.46 (nitroarom. C–O), 150.72, 130.84, 123.53 and 146.28 (nitroarom. C), 146.28 (C-1), 97.31 (C-2), 73.74 and 72.60 (C-3 or C-4), 57.65 (C-5), and 16.63 (C-6).

Anal. Calc. for $C_{13}H_{12}N_4O_5$ (304.26): C, 51.32; H, 3.98; N, 18.41. Found: C, 51.40; H, 3.98; N, 18.37.

*7-*O*-(3-Azido-4-*O*-p-nitrbovenzoyl-2,3,6-trideoxy-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**16**). — The reaction of ε-isoRMN **6** (250 mg, 0.56 mmol) and azido-glycal **15** (300 mg, 0.98 mmol) in the presence of Me_3Si triflate (0.02 g, 0.9 mmol) at −20° as described in general procedure (*a*) afforded **16** (320 mg, 76%), m.p. 209–210°, $[\alpha]_D$ +860° (*c* 0.005), t.l.c. in 5:5:0.5 light petroleum–CH_2Cl_2–EtOAc; ^{1}H-n.m.r. (300 MHz): δ 13.00, 12.81, 12.28 and 12.27 (4s, 4 H, HO-1,4,6,11), 8.26–8.17 (m, 4 H, nitroarom. H), 7.27 (s, 2 H, H-2,3), 5.48 (d, 1 H, $J_{1',2'a}$ 3.4 Hz, H-1'), 5.22 (bs, 1 H, H-7), 4.92 (dd, 1 H, $J_{3',4'}$ 10.0, $J_{4',5'}$ 10.0 Hz, H-4'), 4.27 (s, 1 H, H-10), 4.18 (m, 1 H, H-3'), 4.17 (m, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 4.09 (s, 1 H, HO-9), 3.67 (s, 3 H, CO_2Me), 2.33 (d, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.23 (dd, 1 H, $J_{7,8b}$ 4.4 Hz, H-8b), 1.92–1.80 (m, 2 H, H-2'*a*,2'*e*), 1.85 (m, 1 H, $J_{13,14}$ 7.5, $J_{A,B}$ 15.0 Hz, H-13a), 1.46 (m, 1 H, H-13b), 1.22 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6'), and 1.08 (t, 3 H, H-14).

Anal. Calc. for $C_{35}H_{32}N_4O_{15}$ (748.66): C, 56.15; H, 4.31; N, 7.48. Found: C, 56.07; H, 4.30; N, 7.46.

*7-*O*-(2,3,6-Trideoxy-3-trifluoroacetyl-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**19**). — The treatment of compound **11** (700 mg, 0.855 mmol) according to

general procedure (*b*) yielded **19** (475 mg, 83%), m.p. 216–218°, $[\alpha]_D$ +470° (*c* 0.1), t.l.c. in 95:5:1:0.25:0.1 $CHCl_3$–acetone–AcOH–water–Et_3N; ^{1}H-n.m.r. (300 MHz, $CDCl_3$–MeOD 5:1): δ 7.28 (s, 2 H, H-2,3), 5.36 (d, 1 H, $J_{1',2'a}$ 3.2 Hz, H-1'), 5.14 (d, 1 H, $J_{7,8a}$ 3.0, $J_{7,8b}$ 4.0 Hz, H-7), 4.23 (s, 1 H, H-10), 3.89 (m, 1 H, H-5), 3.86 (m, 1 H, H-3), 3.64 (s, 3 H, CO_2Me), 3.09 (dd, 1 H, $J_{3',4'}$ 9.8, $J_{4',5'}$ 9.8 Hz, H-4'), 2.29 (dd, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.17 (dd, 1 H, H-8b), 2.12 (dd, 1 H, $J_{2'a,2'e}$ 12.6, $J_{2'e,3'}$ 4.6 Hz, H-2'*e*), 1.74 (m, 1 H, $J_{13,14}$ 7, $J_{A,B}$ 15.0 Hz, H-13a), 1.73 (ddd, 1 H, $J_{2'a,3'}$ 9.0 Hz, H-2'*a*), 1.39 (m, 1 H, H-13b), 1.29 (d, 3 H, $J_{5',6'}$ 6.4 Hz, H-6'), and 1.07 (t, 3 H, H-14); *m/z* (f.a.b.) 670 (M + H^+).

Anal. Calc. for $C_{30}H_{30}F_3NO_{13}$ (669.57): C, 53.82; H, 4.52; N, 2.09. Found: C, 53.77; H, 4.54; N, 2.03.

*7-O-(3-Amino-2,3,6-trideoxy-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**20**). — Treatment of compound **11** (700 mg, 0.855 mmol) as described in procedure (*c*) gave **20** (353 mg, 72%), m.p. 173–175°, $[\alpha]_D$ −120° (*c* 0.1, MeOH); t.l.c. in 10:5:2:1:0.5:0.05 $CHCl_3$–acetone–MeOH–AcOH–water–Et_3N; ^{1}H-n.m.r. (300 MHz): δ 7.27 (s, 2 H, H-2,3), 5.48 (d, 1 H, $J_{1',2'a}$ 3.1 Hz, H-1'), 5.20 (d, 1 H, $J_{7,8b}$ 3.6 Hz, H-7), 4.26 (s, 1 H, H-10), 3.97 (m, 1 H, H-5'), 3.73 (s, 1 H, CO_2Me), 3.17 (m, 1 H, H-3'), 3.17 (m, 1 H, H-4'), 2.37 (d, 1 H, $J_{A,B}$ 15.2 Hz, H-8a), 2.25 (dd, 1 H, H-8b), 2.24 (dd, 1 H, H-2'*e*), 1.84 (m, 1 H, $J_{13,14}$ 7.3, $J_{A,B}$ 14.8 Hz, H-13a), 1.82 (ddd, 1 H, H-2'*a*), 1.51 (m, 1 H, H-13b), 1.36 (d, 3 H, $J_{5',6'}$ 6.2 Hz, H-6'), and 1.15 (t, 3 H, $J_{13,14}$ 7.3 Hz, H-14); *m/z* (f.a.b.) 574 (M + H^+).

Anal. Calc. for $C_{28}H_{31}NO_{12}$ (5.73.56): C, 58.64; H, 5.45; N, 2.44. Found: C, 58.38; H, 5.47; N, 2.21.

*7-O-(3-Amino-2,3,6-trideoxy-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**20**). — To a solution of **21** (50 mg, 0.083 mmol) in 4:1 CH_2Cl_2–EtOH (2.5 mL) was added an ethanolic solution (10 mL) containing 4% $NiCl_2 \cdot 6\,H_2O$ and 1% boric acid. The mixture was stirred vigorously, and then $NaBH_4$ (50 mg) was added in portions of ~ 10 mg during 5 h. After 20 h stirring at room temperature, the mixture was filtered through Celite and the filtrate evaporated. Column chromatography (10:5:2:1:0.5:0.05 $CHCl_3$–acetone–MeOH–AcOH–water–Et_3N) on silica gel (15 g) gave **20** (40 mg, 82%). ^{1}H-N.m.r. spectroscopy and l.c. analysis revealed that this compound was identical to compound **20** already described.

*7-O-(3-Azido-2,3,6-trideoxy-α-*L-arabino-*hexopyranosyl)-ε-isorhodomycinone* (**21**). — Treatment of compound **16** (250 mg, 0.33 mmol) according to general procedure (*b*) gave **21** (166 mg, 83%); m.p. 258–260°, $[\alpha]_D$ +1263° (*c* 0.01), t.l.c. in 5:5:2 light petroleum–CH_2Cl_2–EtOAc; ^{1}H-n.m.r. (300 MHz): δ 12.97, 12.81, 12.31 and 12.29 (4s, 4 H, HO-1,4,6,11), 7.25 (s, 2 H, H-2,3), 5.37 (dd, 1 H, $J_{1',2'a}$ 3.8, $J_{1',2'e}$ 1.2 Hz, H-1'), 5.15 (dd, 1 H, $J_{7,8a}$ 1.2 Hz, $J_{7,8b}$ 4.5 Hz, H-7), 4.23 (s, 1 H, H-10), 3.81 (qd, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'}$ 6.5 Hz, H-5'), 3.65 (s, 3 H, CO_2Me), 3.45 (m, 1 H, H-3'), 3.07 (d, 1 H, $J_{3',4'}$ 9.5 Hz, H-4'), 2.27 (dd, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.17 (dd, 1 H, H-8b), 2.10 (ddd, 1 H, $J_{2'a,2'e}$ 13.0, $J_{2'e,3'}$ 4.5 Hz, H-2'*e*), 1.76 (m, 1 H, $J_{13,14}$ 7.5 Hz, $J_{A,B}$ 14.8 Hz, H-13a), 1.63 (ddd, 1 H, $J_{1',2'a}$ 4.0 Hz, $J_{2'a,3'}$ 13.0 Hz, H-2'*a*), 1.39 (m, 1 H, H-13b), 1.29 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6'), and 1.06 (t, 3 H, $J_{13,14}$ 7.5 Hz, H-14).

Anal. Calc. for $C_{28}H_{29}N_3O_{12}$ (559.56): C, 56.09; H, 4.88; N, 7.01. Found: C, 56.13; H, 4.88; N, 6.92.

*7-O-(3-Dimethylamino-2,3,6-trideoxy-α-*L*-arabino-hexopyranosyl)-ε-isorhodomycinone* (**23**). — Treatment of compound **20** (250 mg, 0.435 mmol) according to general procedure (*d*) yielded **23** (226 mg, 83%); m.p. 182–184°, $[\alpha]_D$ +850° (*c* 0.01); t.l.c. in 5:1 $CHCl_3$–MeOH; ^{1}H-n.m.r. (300 MHz): δ 7.29 (s, 2 H, H-2,3), 5.55 (d, 1 H, $J_{1',2'a}$ 3.5 Hz, H-1'), 5.24 (d, 1 H, $J_{7,8a}$ 1.5 Hz, $J_{7,8b}$ 4.5 Hz, H-7), 4.41 (s, 1 H, HO-9), 4.29 (d, 1 H, $J_{8a,10}$ 0.5 Hz, H-10), 3.92 (m, 1 H, $J_{4',5'}$ 9.0, $J_{5',6'}$ 6.5 Hz, H-5'), 3.72 (s, 3 H, CO_2Me), 3.17 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-4'), 2.64 (m, 1 H, $J_{2'e,3'}$ 3.5, $J_{2'a,3'}$ 13.0 Hz, H-3'), 2.39 (dt, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.23 (dd, 1 H, H-8b), 2.20 (s, 6 H, N-Me), 1.91 (dd, 1 H, $J_{2'a,2'e}$ 13.0 Hz, H-2'*e*), 1.85 (m, 1 H, $J_{13,14}$ 7.5, $J_{A,B}$ 15.0 Hz, H-13a), 1.68 (ddd, 1 H, H-2'*a*), 1.45 (m, 1 H, H-13b), 1.38 (d, 1 H, H-6'), and 1.14 (t, 3 H, $J_{13,14}$ 7.5 Hz, H-14); *m/z* (f.a.b.) 602 (M + H^+).

Anal. Calc. for $C_{30}H_{35}NO_{12}$ (601.61): C, 59.89; H, 5.86; N, 2.33. Found: C, 59.76; H, 5.87; N, 2.29.

*7-O-(2,3,6-Trideoxy-3-trifluoroacetylamino-α-*L*-ribo-hexopyranosyl)-ε-isorhodomycinone* (**24**). — Treatment of compound **12** (600 mg, 0.732 mmol) according to general procedure (*b*) gave **24** (426 mg, 87%), m.p. 211–213°, $[\alpha]_D$ +593° (*c* 0.01, MeOH); t.l.c. in 90:10:0.2:0.1 $CHCl_3$–acetone–water–Et_3N, *m/z* (f.a.b.) 676 (M + Li^+), 692 (M + Na^+).

Anal. Calc. for $C_{30}H_{30}F_3NO_{13}$ (669.57): C, 53.82; H, 4.52; N, 2.09. Found: C, 53.72; H, 4.54; N, 2.00.

*7-O-(3-Amino-2,3,6-trideoxy-α-*L*-ribo-hexopyranosyl)-ε-isorhodomycinone* (**25**). — Treatment of compound **12** (720 mg, 0.879 mmol) according to general procedure (*d*) gave **25** (357 mg, 71%), m.p. 146–148°, $[\alpha]_D$ +319° (*c* 0.01), t.l.c. in 10:5:2:1:0.5:0.05 $CHCl_3$–acetone–MeOH–AcOH–water–Et_3N; ^{1}H-n.m.r. (300 MHz, 10:1 $CDCl_3$–MeOD): δ 7.22 (s, 2 H, H-2,3), 5.33 (bs, 1 H, H-1'), 5.00 (bs, 1 H, H-7), 4.10 (s, 1 H, H-10), 4.01 (qd, 1 H, $J_{4',5'}$ 9.8, $J_{5',6'}$ 6.2 Hz, H-5'), 3.74 (s, 3 H, CO_2Me), 3.55 (m, 1 H, H-3'), 3.42 (bs, 1 H, H-4'), 2.26 (d, 1 H, $J_{A,B}$ 15.0 Hz, H-8a), 2.00 (dd, 1 H, $J_{7,8b}$ 4.0 Hz, H-8b), 1.97 (d, 1 H, $J_{2'a,2'e}$ 13.0 Hz, H-2'e), 1.90 (d, 1 H, H-2'*a*), 1.77 (m, 1 H, $J_{13,14}$ 7.5, $J_{A,B}$ 14.8 Hz, H-13a), 1.42 (m, 1 H, H-13b), 1.30 (d, 3 H, $J_{5',6'}$ 6.2 Hz, H-6'), and 1.05 (t, 3 H, H-14); *m/z* (f.a.b.) 574 (M + H^+).

Anal. Calc. for $C_{28}H_{31}NO_{12}$ (573.56): C, 58.64; H, 5.45; N, 2.44. Found: C, 58.27; H, 5.47; N, 2.28.

*7-O-(3-Dimethylamino-2,3,6-trideoxy-α-*L*-ribo-hexopyranosyl)-ε-isorhodomycinone* (**26**). — Treatment of compound **25** (330 mg, 0.575 mmol) according to general procedure (*d*) gave **26** (231 mg, 67%); m.p. 130–135°, $[\alpha]_D$ +540° (*c* 0.01); t.l.c. in 3:1 $CHCl_3$–MeOH; ^{1}H-n.m.r. (300 MHz): δ 13.03, 12.83, 12.34 and 12.34 (4s, 4 H, HO-1,4,6,11), 7.30 (s, 2 H, H-2,3), 5.45 (dd, 1 H, $J_{1',2'a}$ 5.4, $J_{1',2'e}$ 6.6 Hz, H-1'), 5.21 (dd, 1 H, $J_{7,8a}$ 1.2, $J_{7,8b}$ 4.5 Hz, H-7), 4.30 (d, 1 H, $J_{8a,10}$ 1.0 Hz, H-10), 4.08 (dq, 1 H, $J_{4',5'}$ 6.8, $J_{5',6'}$ 6.5 Hz, H-5'), 3.76 (dd, 1 H, $J_{3',4'}$ 4.7 Hz, H-4'), 3.72 (s, 3 H, CO_2Me), 2.76 (m, 1 H, H-3'), 2.59 (s, 6 H, N-Me), 2.39 (br d, 1 H, $J_{A,B}$ 15.1 Hz, H-8a), 2.24 (dd, 1 H, H-8b), 2.08 (ddd, 1 H, $J_{2'a,2'e}$ 13.8, $J_{2'e,3'}$ 3.2 Hz, H-2'*e*), 1.85 (ddd, 1 H, $J_{2'a,3'}$ 11.0 Hz, H-2'*a*), 1.81 (m, 1 H, $J_{13,14}$ 7.3, $J_{A,B}$ 14.8 Hz, H-13a), 1.47 (m, 1 H, H-13b), 1.41 (d, 3 H, H-6'), and 1.14 (t, 3 H, H-14); *m/z* (f.a.b.) 602 (M + H^+).

Anal. Calc. for $C_{30}H_{35}NO_{12}$ (601.61): C, 59.89; H, 5.86; N, 2.33. Found: C, 59.72; H, 5.88; N, 2.30.

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