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A Convergent Ring-Closing Metathesis Approach to Carbohydrate-Based Macrolides with Potential Antibiotic Activity

Petra Blom, Bart Ruttens, Steven Van Hoof, Idzi Hubrecht, and Johan Van der Eycken*

Laboratory for Organic and Bioorganic Synthesis, Department of Organic Chemistry, Ghent University, Krijgslaan 281 (S.4), B-9000 Gent, Belgium

Benedikt Sas, Johan Van hemel, and Jan Vandenkerckhove

Kemin Pharma Europe, Atealaan 4H, B-2200 Herentals, Belgium

johan.vandereycken@ugent.be

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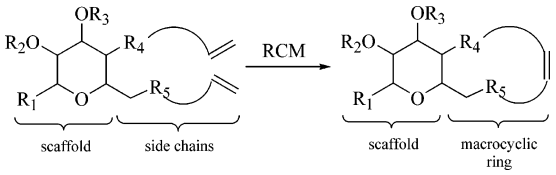

An efficient convergent approach has been developed for the construction of novel, non-natural, carbohydrate-based macrolides. The key step in the synthesis is the formation of the macrocyclic ring via a ring-closing metathesis reaction. The obtained macrolide analogues have been screened for biological activity against Gram-positive and Gram-negative bacteria, including resistant strains, yeasts, and molds.

Because of the intensive use of antibiotics, resistant strains of bacteria have developed, and cross-resistance to different macrolides has been generally observed.[1] This increasing bacterial drug resistance is driving a greater focus on the development of new, efficacious bactericides. We have developed a flexible convergent ring-closing metathesis (RCM) approach for the construction of a new class of molecules consisting of a carbohydrate scaffold fused to a macrocyclic ring system (Figure 1),[2,3] classifying these compounds as macrolides and ketolides.[4]

Carbohydrate substructures often occur in natural macrolides and can contribute significantly to their biological activity. Further, the structural complexity of carbohydrates can be used to introduce wide chemical and stereochemical variation. In addition, as the orientation of the side chains plays an important role in macrocyclizations,[5] one of the functions of the carbohydrate scaffold during the synthesis is to keep the side chains in the correct position. Due to their cyclic nature, thus imposing a conformational restriction, pyranose and furanose sugars are ideally suited for this purpose.

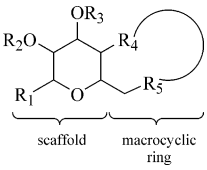

FIGURE 1. General structure of the carbohydrate-based macrolides.

We now report the synthesis of several new carbohydrate-based macrolides and their biological activity against resistant microorganisms. The compounds presented were selected to demonstrate the flexibility of our approach by displaying structural variation in (i) the substituents R_1, R_2, and R_3 on the carbohydrate scaffold, (ii) the type of bond (R_4 and R_5) between the scaffold and the side chains, and (iii) the size of the macrocyclic ring (Figure 1).

Commercially available 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1a**) was chosen as starting material for the preparation of several carbohydrate scaffolds (Scheme 1). $SnCl_4$-catalyzed exchange with thiophenol exclusively gave **1b** (78% yield; $J_{H1-H2} = 10.1$ Hz).[6] Solvolysis of the remaining acetates under Zemplén conditions, followed by acid-catalyzed formation of the 4,6-*O*-benzylidene acetal, afforded **2**[7] (57% from **1a**). Methylation of the free hydroxyl functions of compound **2** gave dimethyl ether **3a**,[8] while benzylation furnished compound **3b**.[7] Acid-catalyzed solvolysis finally provided the scaffolds **4a** and **4b** in 55% and 32% overall yield, respectively. Reductive cleavage of the thiophenyl moiety in **4a** with Raney nickel

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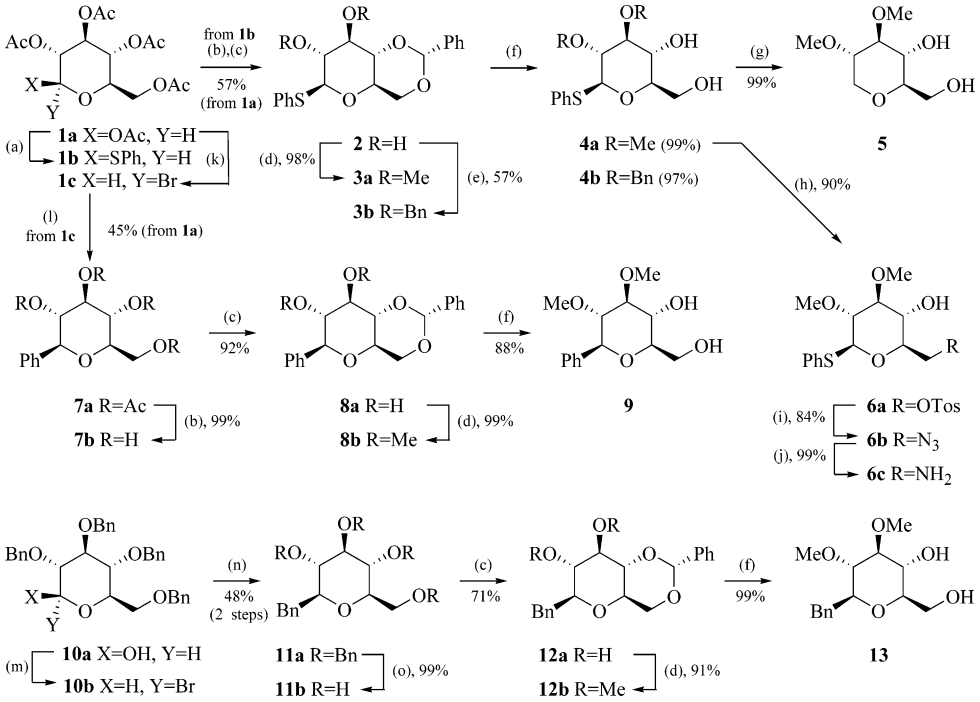
SCHEME 1. Synthesis of the Scaffolds[a]



[a] Reaction conditions: (a) $SnCl_4$, PhSH, CH_2Cl_2, 0 °C to rt, 24 h; (b) K_2CO_3, THF/MeOH (1/1), rt; (c) benzaldehyde dimethyl acetal, 10-CSA, DMF, 110 °C; (d) (i) NaH, DME, 0 °C, 30 min, (ii) MeI, 0 °C to rt; (e) (i) NaH, THF, 0 °C, 30 min, (ii) BnBr, TBAI, 0 °C to rt, 48 h; (f) 10-CSA, MeOH, rt; (g) Raney-Ni, EtOH, rt, 1 h; (h) TsCl, Et_3N, CH_2Cl_2, rt, 24h; (i) NaN_3, DMF, 60 °C, 48h; (j) PPh_3, THF/H_2O (100/1), rt, 48 h; (k) HBr/HOAc (30wt %), rt, 30 min; (l) (i) PhMgBr, Et_2O, 0 °C to rt, 48 h, (ii) Ac_2O, pyridine, rt, 18 h; (m) oxalyl bromide, CH_2Cl_2/DMF (20/1), rt, 1 h; (n) BnMgBr, Et_2O, 0 °C to rt, 18 h; (o) H_2, Pd/C, EtOH, 3−4 atm, rt, 2 h.

in absolute ethanol afforded scaffold **5** nearly quantitatively.[9] Regioselective tosylation of **4a**, followed by nucleophilic displacement of tosylate **6a** with sodium azide and Staudinger reduction of the resulting azide **6b**, gave amino alcohol **6c** (75% from **4a**).[10]

Glucopyranosyl bromide **1c** (J_{H1-H2} = 4.0 Hz) was converted to **7a** (J_{H1-H2} = 9.9 Hz) following a literature procedure.[11] Zemplén methanolysis (→ **7b**), introduction of the 4,6-*O*-benzylidene acetal (→ **8a**), methylation (→ **8b**), and finally, 10-CSA-mediated solvolysis of the acetal furnished scaffold **9** (36% from **1c**). Intermediate **11a** (J_{H1-H2} = 9.2 Hz) was prepared from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**10a**) by S_N2 substitution of α-bromide **10b** (J_{H1-H2} = 3.7 Hz) with benzylmagnesium bromide, following the procedure described by Panigot and Curley.[12] Catalytic hydrogenolysis of the benzyl ethers furnished tetrol **11b**. A sequence of reactions identical to that for the preparation of compound **9** from **7b** gave scaffold **13** (30% from **10a**).

Scaffolds **4a**, **4b**, **5**, **6b**, **6c**, **9**, and **13** were used to prepare a variety of macrolide analogues. Due to the substitution of the anomeric oxygen by hydrogen (**5**), sulfur (**4a**, **4b**, **6b**, **6c**) and carbon (**9**, **13**) an improved metabolic stability as compared to natural carbohydrates can be expected.[13] Furthermore, the thiophenyl substituent offers the possibility for additional differentiation at the C1 position by glycosidation with other sugars, alcohols or nucleophiles.[14] While the abundant appearance of methoxy groups in natural macrolides prompted us to focus our attention on methyl ethers at the C2 and C3 position of the scaffolds, we also explored several other functional groups at these positions including benzyl ethers (**4b**). Finally, the (latent) amino function at position C6 in scaffolds **6b** and **6c** enabled us to investigate different types of bonds between scaffold and side chains. A simple two-step reaction sequence, consisting of the attachment of the side chains and subsequent RCM, is sufficient to convert the scaffolds to carbohydrate-based macrolide analogues. Initially, the efficiency of this convergent approach toward macrolide analogues was evaluated using simple, commercially available bifunctional aliphatic chains. Macrocyclic glycolipids such as Woodrosin I and Tricolorin A also contain a long hydrophobic hydrocarbon chain bridging two sugar hydroxyl groups and show interesting biological activities.[3d] The synthesis of highly functionalized macrolides from carbohydrate scaffolds and acyclic carbohydrate side chains is currently in progress and will be reported elsewhere.

Attachment of 4-pentenoyl chloride to scaffold **4a** provided precursor **14** in 83% yield (Scheme 2). With **4b**, the same reaction conditions led to an inseparable mixture of compound **21** and unknown side products. In situ activation of 4-pentenoic acid with 1,3-diisopropyl-

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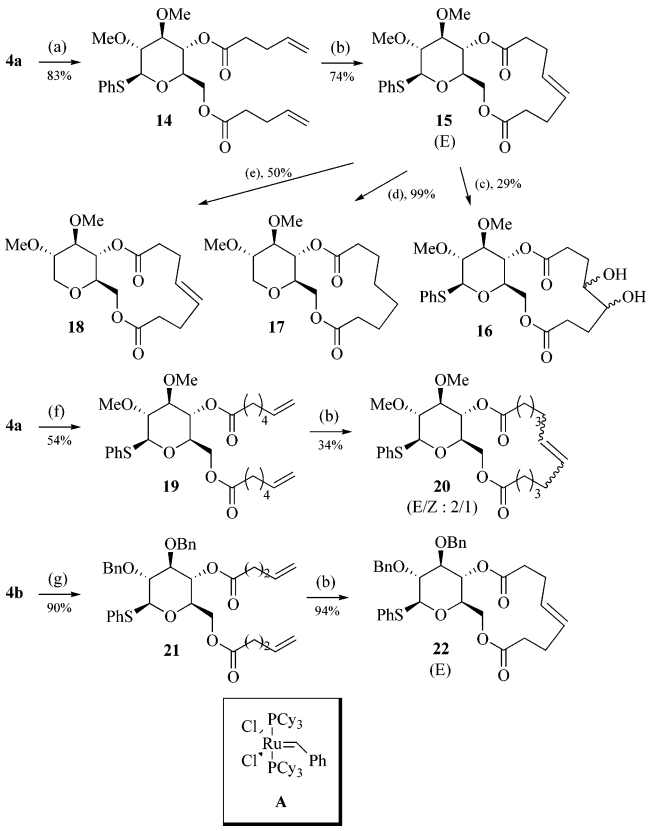
SCHEME 2. Macrolide Analogues Based on Ester Bonds between Scaffold and Side Chains[a]

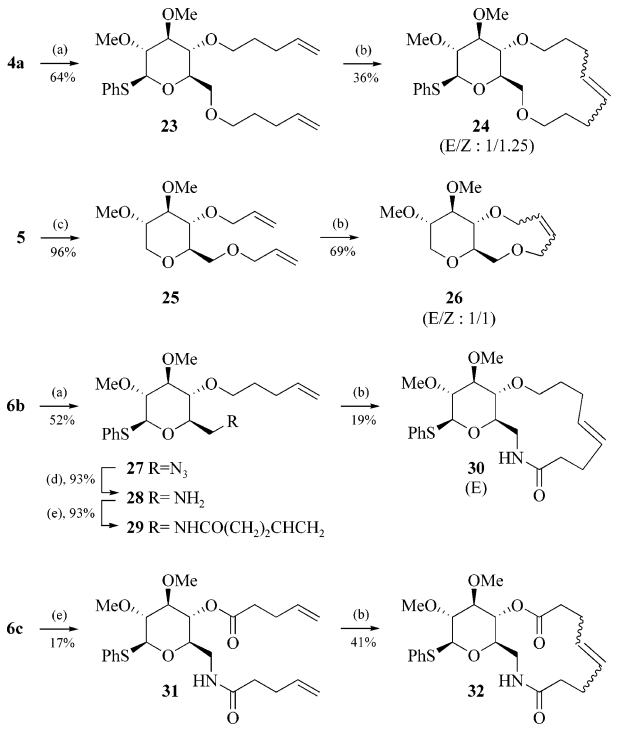


[a] Reaction conditions: (a) 4-pentenoyl chloride, pyridine, DMAP, CH_2Cl_2, rt, 18 h; (b) Grubbs' catalyst (**A**) (10 mol %), CH_2Cl_2, rt; (c) OsO_4, NMO, *t*-BuOH, acetone/H_2O (2.5/1), rt, 1 h; (d) Raney Ni, H_2, EtOH, rt, 1 h; (e) Raney Ni, EtOH, rt, 1 h; (f) 6-heptenoic acid, DIC, HOBt, DMAP, CH_2Cl_2, rt, 72 h; (g) 4-pentenoic acid, DIC, HOBt, DMAP, CH_2Cl_2, rt, 120 h.

carbodiimide (DIC) in the presence of 1-hydroxybenzotriazole (HOBt), and DMAP proved to be more effective and furnished diester **21** in excellent yield. Likewise, reaction of scaffold **4a** with 6-heptenoic acid gave diene **19**. Macrocyclization of **14**, **19**, and **21** via a RCM reaction with Grubbs' first generation catalyst (**A**)[5] gave fair to good yields of the desired macrolide analogues **15**, **20** and **22**, respectively. A remarkable selectivity was observed in the case of compounds **15** and **22**, where the vicinal coupling constants of the vinylic protons indicated exclusive formation of a double bond with *E*-configuration (J = 15.0 and 14.8 Hz, respectively). In contrast, the larger macrocycle **20** was obtained as a 2/1 mixture of the *E*- and *Z*-alkene. It should be pointed out that the yield for ring closure of compound **19** (34%) refers to the isolated yield of pure *E*- and *Z*-isomers and neglects a significant mixed fraction.

Next, we explored the derivatization of the double bond. Macrolide **15** was readily hydrogenated with Raney nickel under hydrogen atmosphere with concomitant reductive cleavage of the anomeric thiophenyl group, affording **17**. Application of the same catalyst under inert atmosphere afforded compound **18** in moderate yield. Direct dihydroxylation of **15** with 4-methylmorpholine *N*-oxide (NMO) and a catalytic amount of OsO_4 furnished a diastereomeric mixture of the corresponding *syn*-diols

SCHEME 3. Different Bond Types between Scaffold and the Macrocyclic Ring[a]



[a] Reaction conditions: (a) 5-bromo-1-pentene, NaH, TBAI, THF, 0 °C to rt; (b) Grubbs' catalyst (**A**) (10 mol %), CH_2Cl_2, rt; (c) allyl bromide, NaH, TBAI, THF, 0 °C to rt, 18 h; (d) PPh_3, THF/H_2O (9/1), rt, 48 h; (e) 4-pentenoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C to rt.

16 in 29% yield (not optimized).[15] Epoxidation failed due to the sensitivity of the thiophenyl moiety to oxidation.

Some examples of different bond types between scaffold and the macrocyclic ring are shown in Scheme 3. Scaffolds **4a**, **5**, and **6b** were efficiently alkylated with side chains to provide compounds **23**, **25**, and **27**. Staudinger reduction of azide **27** furnished amine **28**,[10] easily acylated with 4-pentenoyl chloride under basic conditions to give the mixed diene **29**. Due to excessive formation of side products the same acylation conditions afforded only low yields of compound **31** when applied to **6c**. The moderate yields for cyclization of dienes **23**, **25**, **29** and **31** can be attributed to the linkage between scaffold and alkene side chains. RCM is one of the most efficient entries into large rings provided that a few basic parameters are properly assessed.[5,16] Apart from the obvious spatial requirements, the presence of polar groups for coordination with the metal carbene complexes of the catalytic cycle and their orientation relative to the alkene units are particularly crucial for the success of RCM-based macrocyclizations. Compared to esters, ethers possess only a moderate capacity to coordinate with the metal carbene intermediates, resulting in a low yield of macrolide **24**.

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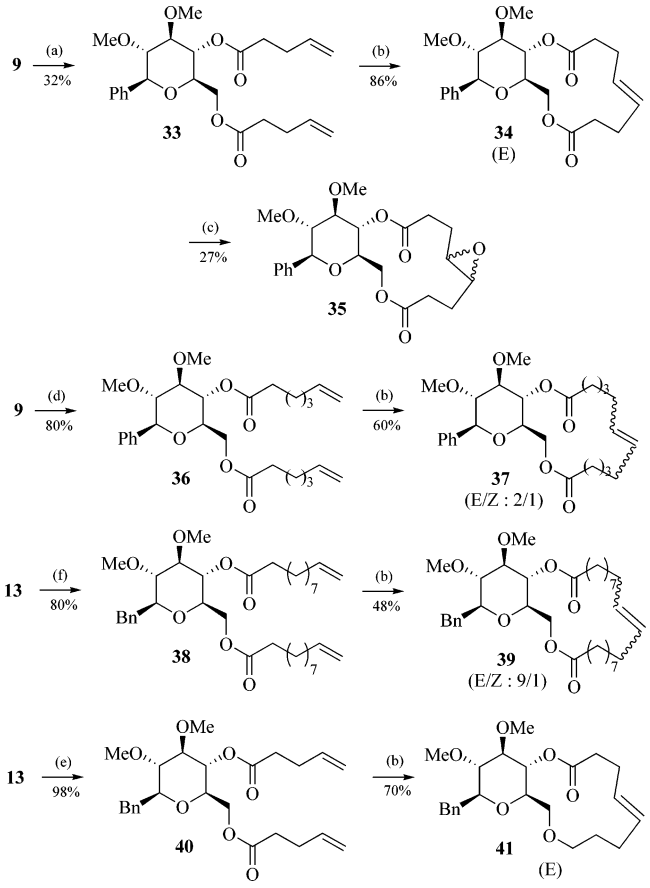
TABLE 1. Antibacterial and Antifungal Activity of Some of the Macrolides

	% of growth at a dose of 25 ppm compared to the negative control							
compd	*E. faecalis*	VRE	*S. aureus*	MRSA	*P. aeruginosa*	*S. typhimurium*	*C. albicans*	*M. gypseum*
control	100	100	100	100	100	100	100	100
20 (*E*)	76.5	61.8	102.8	114.6	29.0	76.4	98.4	74
20 (*Z*)	98.4	81.0	97.0	104.1	79.8	87.0	85.0	58
24 (*E*)	87.5	82.0	98.3	107.3	91.6	90.1	93.9	60
24 (*Z*)	85.5	86.4	100.4	110.8	67.6	90.1	103.2	54
32	68.2	93.0	56.7	75.6	83.3	96.6	78.1	81

SCHEME 4. Synthesis of Macrolide Analogues with Different Ring Sizes[a]



[a] Reaction conditions: (a) 4-pentenoyl chloride, pyridine, DMF, 90 °C, 2 h; (b) Grubbs' catalyst (**A**) (10 mol %), CH_2Cl_2, rt; (c) *m*CPBA, CH_2Cl_2, rt, 72 h; (d) 6-heptenoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C to rt, 18 h; (e) 4-pentenoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C to rt, 22 h; (f) 10-undecenoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C to rt, 18 h.

The much better yield for the formation of compound **26** probably originates from the close spatial arrangement of the shorter side chains, making cyclization entropically less disfavorable. In contrast, the poor yields of dienes **30** and **32** in the RCM are probably the result of the strong chelation of the amide bonds to the metal carbene complex, sequestering the catalyst in an unproductive form. Both cycloalkenes **24** and **26** were formed roughly as 1/1 mixtures of *E*- and *Z*-isomers. Macrolide analogue **30** was obtained exclusively as the *E*-alkene (J = 14.9 Hz). In the case of compound **32**, line broadening of the vinylic signals, caused by the hindered rotation around the amide bond, obstructs proper determination of the *E*/*Z* ratio.

Depending on the choice of the side chains, macrocyclic rings of different sizes can be attached to the carbohydrate scaffolds (Scheme 4). All diene intermediates **33**, **36**, **38** and **40** were easily prepared by acylation of scaffolds **9** and **13** with alkenoyl chlorides under basic conditions, although formation of side products at elevated temperature decreased the yield of diester **33** significantly. Subsequent RCM with Grubbs' catalyst (**A**) of the diene precursors **33**, **36**, **38** and **40** yielded the desired macrocycles **34**, **37**, **39** and **41**, respectively. Epoxidation of substrate **34** with 3-chloroperbenzoic acid (mCPBA) afforded a diastereomeric mixture of epoxides **35** in 27% yield (not optimized).[17]

The biological activity of the macrolide analogues towards Gram-positive bacteria (*Enterococcus faecalis,* vancomycin-resistant enterococci (VRE), *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA)), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Salmonella typhimurium*), a typical yeast (*Candida albicans*), and a typical mold (*Microsporum gypseum*) were evaluated. Some results are summarized in Table 1. *E*-**20** has a good overall activity against gram-negative bacteria, especially against *P. aeruginosa* and to a lesser extent against VRE. Compound **32** displays good activity against gram-positive bacteria, including resistant strains such as MRSA. Macrolide *Z*-**24** shows the best activity against *M.* gypseum.

In conclusion, a highly convergent approach for the synthesis of novel, non-natural, carbohydrate-based macrolide analogues has been developed. Some of the macrolides and intermediates display moderate antibiotic activity against Gram-positive and Gram-negative bacteria and fungi. Currently, lead optimization and preparation of highly functionalized macrolides are under investigation.

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Supporting Information Available: Synthesis and spectroscopic data of all synthesized compounds, full experimental screening protocols and results, and theoretical calculations of the *E*/*Z* ratios based on molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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