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Synthesis of bi- and tricyclic analogues of *myo*-inositol 3,4,5,6- and 1,4,5,6-tetrakisphosphate with extended carbon backbone

Andrew Schnaars[a] and Carsten Schultz[a,b,*]

[a]*Institut für Organische Chemie,UFT, Universität Bremen, Leobener Str., 28359 Bremen, Germany*
[b]*Max-Planck-Institut für molekulare Physiologie, Otto-Hahn-Str. 11, 44227 Dortmund, Germany*

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Abstract—*myo*-Inositol 3,4,5,6-tetrakisphosphate [Ins(3,4,5,6)P_4] and its enantiomer exhibit distinct functions in the regulation of transepithelial chloride secretion. In search for agonists and antagonists of these potential messengers we synthesized a bicyclic oxepin analogue and a tricyclic analogue with a selfprotecting dioxolane moiety. The carbon backbone extension was linked to the C2 position. Ring closure was achieved by a metathesis reaction.

1. Introduction

myo-Inositol 3,4,5,6-tetrakisphosphate [Ins(3,4,5,6)P_4, **1**] and *myo*-inositol 1,4,5,6-tetrakisphosphate [Ins(1,4,5,6)P_4, **2**] belong to the signalling cascade of higher phosphorylated inositol polyphosphates and are biosynthesized from *myo*-inositol 1,3,4,5,6-pentakisphosphate upon receptor stimulation.[1] In human epithelial cells, Ins(3,4,5,6)P_4 was shown to act as a negative regulator of transepithelial chloride secretion by uncoupling the latter from the calcium signal.[2] Therefore, high intracellular levels of Ins(3,4,5,6)P_4 will limit chloride secretion via calcium-activated chloride channels.[2,3] These are of particular therapeutic interest in cases where chloride secretion through other channels is defect, namely in cystic fibrosis.[4] Another negative regulator of chloride secretion in epithelial cells is the phospholipid phosphatidylinositol 3,4,5-trisphosphate[5] which is generated through the activation of thyrosine kinase receptors by growth factors like EGF or TGFα.[6] It was shown that artifically elevated levels of Ins(1,4,5,6)P_4 blocked the EGF-induced inhibition of chloride secretion[7] and is suspected to be involved in regulating transcription.[8] We are therefore searching for compounds that might act as Ins(3,4,5,6)P_4 antagonists and Ins(1,4,5,6)P_4 agonists (Fig. 1).

Here we describe the synthesis of analogues of **1** and **2** with an extended carbon backbone forming the bi- and tricyclic derivatives **3** and **4**. Since the compounds are prepared as racemates, they may serve as Ins(3,4,5,6)P_4 antagonists as well as Ins(1,4,5,6)P_4 agonists in future biological experiments.

2. Results and discussion

For the extension of the inositol carbon backbone we chose the C2 carbon because previous alterations at the 2-OH position had no negative effect on biological activity.[9]

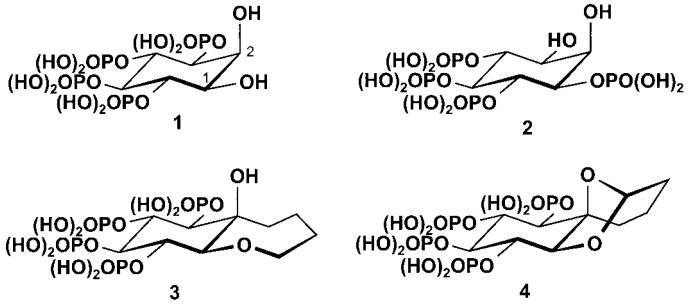

Figure 1. Structures of *myo*-inositol 3,4,5,6- and 1,4,5,6-tetrakisphosphate and derivatives with added ring structures, shown as 3,4,5,6-tetrakisphosphate analogue enantiomers.

Keywords: cyclitols; inositol phosphates; ring-closure metathesis.
* Corresponding author. Tel.: +49-231-133-2436; fax: +49-231-133-2699; e-mail: carsten.schultz@mpi-dortmund.mpg.de

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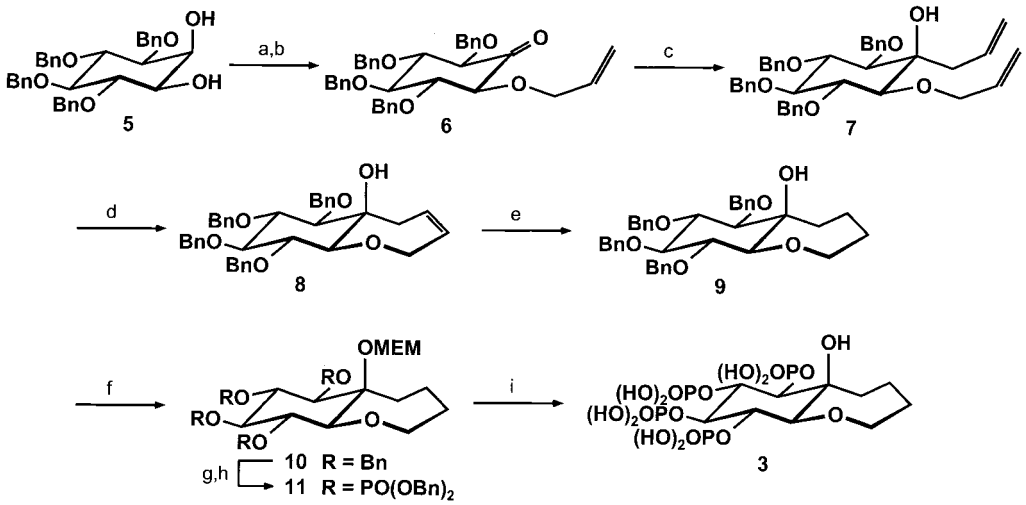
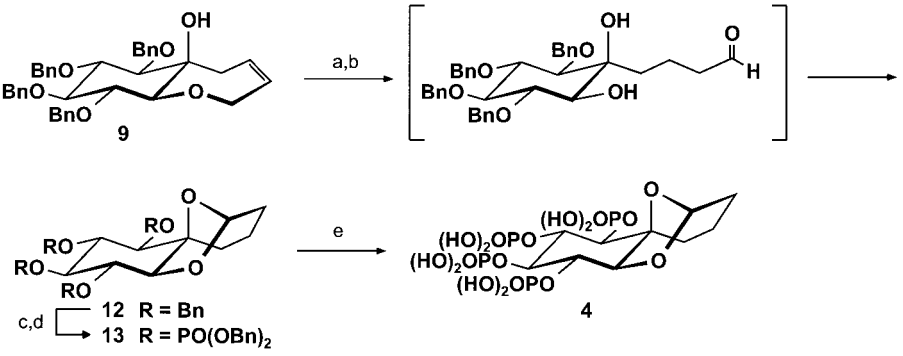


Scheme 1. *Reagents and conditions*: (a) Bu_2SnO, reflux, then CsF, Allyl-Cl, rt, 78%; (b) Ac_2O, DMSO, 79%; (c) Allyl-MgCl in THF, Et_2O, 63%; (d) $(Cy_3P)_2Ru(IV)CHPhCl_2$ (1.1 mol%), CH_2Cl_2, 94%; (e) $(Ph_3P)_3Ru(II)Cl_2$ (7 mol%), MeOH, reflux, then $NaBH_4$, 4 h, later 1% TFA in MeCN, rt, 63%; (f) NaH, DMF, MEM-Cl after 12 h, 86%; (g) H_2/Pd–C (10%), ethanol, 1% iPr$_2$NEt; (h) $(BnO)_2PNiPr_2$, tetrazole, MeCN, then MeCOOOH (32% in acetic acid), 56% (for two steps); (i) H_2/Pd–C (10%), ethanol, finally Dowex 50 WX8, H^+-form, 92%.

Ring structures were aimed for to provide conformational rigidity, which can be accomplished for instance by annulation of seven-membered rings or tricyclic structures to the inositol backbone. Seven-membered rings are readily accessible by olefin metathesis as outlined below. The latter requires the introduction of two olefins, one connected to the 1-hydroxyl group and one to the C2-carbon, as key steps.

The common precursor *rac*-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**5**) was regioselectively alkylated with allyl bromide at the 1-hydroxyl group as described before.[10] Swern oxidation furnished inosose **6** which was subsequently treated with allyl magnesium chloride in THF. The reaction gave predominantly the equatorially alkylated inositol derivative **7** (63%) although 18% of the axially substituted isomer could be isolated by preparative HPLC. Ring closure was accomplished by addition of Grubb's catalyst (1.1 mol%).[11] The desired octahydro-benzo[*b*]oxepin **8** was purified by preparative-scale HPLC (RP-18, 50×250 mm, 40 mL/min, 90% MeOH) and was obtained in excellent yield (94%) as a colorless solid (Scheme 1).

The reduction of the homoallylic ether **8** turned out to be a difficult task. Finally **8** was reduced to the decahydro-benzo[*b*]oxepin **9** (63% yield) with catalytic amounts of dichloro tris(triphenylphosphine) ruthenium(II) (7 mol%) and sodium borohydride (42 mol%), thus making use of the undesired and greatly avoided hydrogenation side-reaction described by Frauenrath and co-workers.[12] The reduction was presumably directed by the axial hydroxyl group and turned out to be surprisingly efficient with respect to the amount of reducing agent required. Apart from **9** smaller amounts (25%) of an enol ether resulting from migration of the double bond were isolated. To avoid inseparable mixtures the crude products were treated with trifluoroacetic acid to hydrolyze the enol ether and give the tricyclic compound **12** (see below). **9** was subsequently alkylated with 2-methoxy ethoxymethyl chloride (MEM-Cl) at the 2-hydroxyl group to give the fully protected compound **10**. Due to the lability of the MEM group, catalytic hydrogenolysis (Pd/C 10%) of the benzyl ethers was performed in the presence of small amounts of di-*iso*-propylethylamine. Phosphorylation of the intermediate tetrol was achieved by standard treatment with dibenzyl *N*,*N*-di-*iso*-propyl phosphoramidite in the presence of tetrazole and subsequent oxidation with peracetic acid.



Scheme 2. *Reagents and conditions*: (a) $(Ph_3P)_3Rh(I)Cl$, 90% ethanol, reflux; (b) 2% TFA in $CHCl_3$, 3d, 56% (for two steps); (c) H_2/Pd–C(10%), ethanol; (d) $(BnO)_2PNiPr_2$, tetrazole, MeCN, then MeCOOOH (32% in acetic acid), 51% (for two steps); (e) H_2/Pd–C(10%), ethanol, 82%. A sample was converted to the sodium salt by treatment with Dowex 50 WX8, sodium form.

Complete deprotection of the fully protected tetrakisphosphate **11** gave the desired (5aα,6α,7β,8α,9aα)-6,7,8,9-tetrakis(phosphonooxy)-5a-hydroxyl-decahydrobenzo[*b*]-oxepin (**3**).

Preliminary experiments for the reaction of **6** with a vinyl reagent showed little selectivity for the Grignard reaction but eventually gave the desired octahydrochromene tetrakisphosphate derivative with an annulated six-membered ring (not shown) (Scheme 2).

To pursue the preparation of the tricyclic compound **4**, the octahydro-benzo[*b*]oxepin **8** was treated with tris(triphenylphosphin)rhodium(I) chloride in aqueous ethanol (90%) followed by the acidic hydrolysis with trifluoroacetic acid of the enol ether. The resulting aldehyde immediately cyclized to give the dioxatricyclo-[6.3.1.$0^{1,6}$]-dodecane **12**. Hence the aldehyde group, a potential linker group for binding the inositol phosphates to resins etc, represents an intramolecular protecting group for the two hydroxyl groups. As a minor by-product (10% yield) the reduced decaoctahydro-benzo[*b*]oxepin **9** was isolated. Hydrogenolysis of **12** under neutral conditions and subsequent phosphorylation gave the fully protected tetrakisphosphate in 59% overall yield. Deprotection by yet another hydrogenolysis afforded the desired product (1*S*,2*R*,3*S*,4*R*,5*S*,6*S*,8*R*)-2,3,4,5-tetrakis-(phosphonooxy)-7,12-dioxatricyclo-6.3.1.$0^{1,6}$]-dodecane (**4**) in 82% yield.

In conclusion, we here present the first synthesis of Ins(3,4,5,6)P_4 and Ins(1,4,5,6)P_4 analogues with altered carbon backbone. Biological experiments with compounds **3** and **4** with epithelial cells are currently under way and the results will be published elsewhere in due course.

3. Experimental

3.1. General methods

Melting points: Büchi B-540 apparatus (uncorrected). ^{1}H NMR spectra: 200 MHz, Bruker DTX 200, TMS as internal standard. ^{31}P NMR spectra: 81 MHz, 85% H_3PO_4 as external standard. Chemical shifts are reported as usual in ppm units. Mass spectra: Finnigan MAT 8222 mass spectrometer with fast atom bombardment (FAB) ionisation or direct chemical ionisation (DCI) with NH_3 as ionisation gas, as indicated. High resolution masses were determined relative to known compounds with mass not differing by more than 10%. Optical rotations were measured at the sodium *D*-line in a 10 cm cell with a Perkin–Elmer 1231 polarimeter. Ultrafiltration of the palladium/carbon catalyst was performed with a Sartorius filtration apparatus SM 162 01 using filters from regenerated cellulose (Sartorius, SM 116 04). Elemental analysis was performed by Mikroanalytisches Labor Beller, Göttingen, Germany. Analytical HPLC was performed on a LDC/Milton Roy ConstaMetric III pump with a LDC/Milton Roy UV Monitor D detector (254 nm) or a Knaur refractive index detector. The analytical column was a 250×4 mm steel tube filled with RP18 material (Merck, LiChrosorb, 10 μm). Preparative HPLC was performed using a Shimadzzu LC 8A pump with a preparative scale LDC UV III monitor detector (254 nm) and a Merck Prepbar steel column (250×50 mm) filled with RP18 material (LiChrospher 100, 10 μm). The eluents were methanol-water mixtures; compositions are given in % methanol (MeOH).

All reagents were obtained in the highest purity available. Where necessary, solvents were dried and/or distilled before use. All dry solvents were stored over molecular sieves. *rac*-3,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (**5**) and its 1-*O*-allyl ether were prepared according to procedures described previously.[10]

3.1.1. *rac*-1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inosose (6). 2.16 g (3.75 mmol) 1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol was dissolved in 10 mL DMSO and 1.5 mL acetic anhydride (15.9 mmol) was added under an argon atmosphere. After 48 h at room temperature all volatile ingredients were removed in high vacuum and the crude yellow residue was recrystallized twice from methanol to give 1.70 g (3.0 mmol, 79% yield) of **6**, mp 124–125°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.53–7.23 (m, 20H, Ph), 6.14–5.90 (m, 1H, $OCH_2CH{=}CH_2$), 5.47–5.20 (m, 2H, $OCH_2CH{=}CH_2$), 5.06–4.57 (m, 8H, OCH_2Ph), 4.49–4.34 (m, 1H, $OCHHCHCH_2$), 4.24 (dd, 1H, ^3J=9.9 Hz, 1.6 Hz, H-1), 4.21–4.07 (m, 1H, $OCHHCHCH_2$), 4.16 (dd, 1H, ^3J=9.9 Hz, 1.6 Hz, H-3), 3.95 (dd, 1H, ^3J=9.1 Hz, H-5), 3.69 (dd, 1H, ^3J=9.7 Hz, H-4), 3.66 (dd, 1H, ^3J=9.6 Hz, H-6); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 203.44 (C), 139.37 (C), 139.37 (C), 139.31 (C), 139.14 (C), 135.83 (CH), 129.18–128.21 (CH, Bn) 117.25 (CH_2), 84.46 (CH), 84.30 (CH), 82.15 (CH), 81.57 (CH), 81.51 (CH), 75.79 (CH_2), 75.50 (CH_2), 75.50 (CH_2), 73.03 (CH_2), 72.16 (CH_2); DCI-MS (NH_3), *m/z* (%), 579 [$M+H^+$] (10), 489 [$M+2H^+-Bn^+$] (68), 399 [$M+3H^+-2Bn^+$] (6), 108 [Bn^++NH_3] (100); Anal. Calcd for $C_{37}H_{38}O_6$ (578.69); C, 76.79; H, 6.61. Found: C, 76.54; H, 6.53.

3.1.2. *rac*-(1*S*,2*R*,3*S*,4*R*,5*S*,6*S*)-1-Allyl-6-allyloxy-2,3,4,5-tetrakis(benzyloxy)-1-hydroxycyclohexane (7). 6 (1.54 g, 2.67 mmol) was suspended in 25 mL dry diethyl ether and the mixture was cooled to −25°C; 1.51 mL (3.03 mmol) of a 2-molar solution of allyl-magnesium chloride in THF was added under argon and vigorous stirring. After 20 min another 0.3 mL (0.6 mmol) allyl magnesium chloride solution was added. The mixture was allowed to warm up and was quenched with 20 mL, 0.5 M phosphate buffer, pH 7, after 14 h. The organic layer was diluted with *tert*-butyl methyl ether, extracted with brine and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent in vacuum the solid residue was chromatographed by preparative HPLC (RP-18, 40 mL/min, 90% MeOH, t_{Ret}=34.50 min) to give 1.04 g (1.68 mmol, 63% yield) **7** as a colorless solid, mp 97–98°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51–7.29 (m, 20H, Ph), 6.19–5.95 (m, 1H, $OCH_2CH{=}CH_2$), 5.92–5.68 (m, 1H, $CCH_2CH{=}CH_2$), 5.44–5.15 (m, 4H, $C{=}CH_2$), 5.18–4.73 (m, 8H, CH_2Ph), 4.60 (dd, 1H, ^2J=12.0 Hz, ^3J=5.7 Hz, $OCHHCH{=}CH_2$), 4.27 (dd, 1H, ^2J=12.1 Hz, ^3J=5.7 Hz, $OCHHCH{=}CH_2$), 4.15 (dd, 1H, ^3J=9.4 Hz, H-3), 4.10 (dd, 1H, ^3J=9.4 Hz, H-5), 3.60 (dd, 1H, ^3J=9.8 Hz, H-4), 3.50 (d, 1H, ^3J=9.5 Hz, H-6), 3.37 (d, 1H, ^3J=9.4 Hz, H-1), 2.72 (d, 2H, ^3J=7.2 Hz, $CCH_2CH{=}CH_2$), 2.39 (br s, 1H, O*H*); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ=139.08 (C), 139.08 (C),

138.96 (C), 138.65 (C), 135.34 (CH), 133.33 (CH), 129.18–127.51 (CH, Bn), 119.92 (CH_2), 117.39 (CH_2), 83.69 (CH), 83.55 (CH), 83.55 (CH), 80.43 (CH), 80.17 (CH), 78.02 (C), 76.31 (CH_2), 76.31 (CH_2), 76.31 (CH_2), 75.87 (CH_2), 75.17 (CH_2), 39.94 (CH_2); FAB-MS (NBA), *m/z* (%), 621 [$M+H^+$] (3), 91 [Bn^+] (100), 772 [$M+NBA-H^+$] (1), 619 [$M-H^+$] (1), 503 [$M-BnO^+$] (14); Anal. Calcd for $C_{40}H_{44}O_6$ (620.79); C, 77.39; H, 7.14. Found: C, 77.21; H, 7.04.

3.1.3. *rac*-(5aα,6α,7β,8α,9β,9aα)-6,7,8,9-Tetrakis(benzyloxy)-5a-hydroxy-2,5,5a,6,7,8,9,9a-octahydrobenzo[*b*]oxepin (8)7 (971 mg, 1.57 mmol) was dissolved in 140 mL dry methylene chloride; 14 mg (17 μmol) bis(tricyclohexylphosphine)benzylidene-ruthenium(IV)-dichloride was added under an argon atmosphere. The solution was stirred for 72 h in the dark. The organic layer was diluted with methylene chloride and washed several times with phosphate buffer pH 7 and brine. After drying over sodium sulfate and filtration, the organic layer was dried and the crude residue was purified by preparative HPLC (RP-18, 40 mL/min, 90% MeOH, t_{Ret}=25.25 min) to give 875 mg (1.49 mmol, 94% yield) **8** as a colorless solid, mp: 123–124°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48–7.23 (m, 20H, Ph), 6.09–5.95 (m, 1H, H-3), 5.80–5.65 (m, 1H, H-4), 5.17–4.67 (m, 8H, OCH_2Ph), 4.50 (dd, 1H, ^2J=15.4 Hz, ^3J=5.6 Hz, H-2$_a$), 4.25–4.07 (m, 1H, H-2$_b$), 4.12 (dd, 1H, ^3J=9.6 Hz, H-7), 3.87 (dd, 1H, ^3J=9.2 Hz, H-9), 3.60 (dd, 1H, ^3J=9.5 Hz, H-8), 3.51 (d, 1H, ^3J=9.2 Hz, H-9a), 3.27 (d, 1H, ^3J= 9.7 Hz, H-6), 2.97 (dd, 1H, ^2J=15.8 Hz, ^3J=7.8 Hz, H-5$_a$), 2.64 (s, 1H, O*H*), 2.22–2.07 (m, 1H, H-5$_b$); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 139.46 (C), 139.16 (C), 139.02 (C), 138.35 (C), 132.33 (CH), 129.00–127.95 (CH, Bn),128.33 (CH), 88.53 (CH), 83.36 (CH), 82.79 (CH), 82.74 (CH), 82.36 (CH), 76.64 (*C*H_2), 76.64 (*C*H_2), 76.34 (CH_2), 76.30 (CH_2), 74.29 (C), 69.72 (CH_2), 37.42 (CH_2); FAB-MS (NBA), *m/z* (%), 593 [$M+H^+$] (1), 91 [Bn^+] (100), 745 [$M+NBA^-$] (2), 591 [$M-H^+$] (<1); Anal. Calcd for $C_{38}H_{40}O_6$ (592.73); C, 77.00; H, 6.80. Found: C, 77.03; H, 6.73.

3.1.4. *rac*-(5aα,6α,7β,8α,9β,9aα)-6,7,8,9-Tetrakis(benzyloxy)-5a-hydroxydecahydrobenzo[*b*]oxepin (9). 8 (684 mg, 1.16 mmol) and dichloro tris(triphenylphosphine) ruthenium(II) (81 mg, 85 μmol) were suspended in 18 mL degassed methanol under argon. The mixture was heated to reflux in the dark. After the solution turned homogeneous 18 mg sodium borohydride (0.48 mmol) was added and the solution was held under reflux for another four hours. After cooling to room temperature the solution was stirred for another 12 h and finally dried under reduced pressure. The residue was resuspended in 7.5 mL acetonitrile and 10 mL CH_2Cl_2. Then 0.14 mL trifluoroacetic acid were added and the mixture was stirred for 5 days at room temperature. The reaction was quenched by adding 0.3 mL di-*iso*-propylethylamine. All volatile components were removed under reduced pressure and the dry residue was dissolved in *tert*-butylmethyl ether, extracted with phosphate buffer (pH 7) and brine. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated. Preparative HPLC (RP-18, 84% MeOH, 40 mL/min, t_{Ret}=41.10 min) gave 433 mg (0.73 mmol, 63% yield) of **9** as a white solid, mp 130°C, and 170 mg (0.29 mmol, 25% yield) of the tricyclic compound **12**. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59–7.22 (m, 20H, Ph), 5.09–4.66 (m, 8H, CH_2Ph), 4.10 (dd, 1H, ^3J=9.6 Hz, H-9), 4.03–3.84 (m, 2H, OCH_2–oxepane), 3.01 (dd, 1H, ^3J=9.1 Hz, H-7), 3.59 (d, 1H, ^3J=9.6 Hz, H-8), 3.39 (d, 1H, ^3J=9.2 Hz, H-6), 3.29 (d, 1H, ^3J=9.2 Hz, H-9a), 2.57 (s, 1H, O*H*), 2.41–2.24 (m, 1H, oxepane), 2.13–1.59 (m, 4H, oxepane), 1.46–1.26 (m, 1H, oxepane); ^{13}C NMR ($CDCl_3$) δ 139.47 (C), 139.26 (C), 139.19 (C), 138.62 (C), 129.11–128.07 (CH, Bn), 84.25 (CH), 83.41 (CH), 83.01 (CH), 82.84 (CH), 82.27 (CH), 76.78 (CH_2), 76.74 (CH_2), 76.43 (C), 76.42 (CH_2), 76.37 (CH_2), 69.79 (CH_2), 41.11 (CH_2), 30.03 (CH_2), 19.74 (CH_2); DCI-MS (NH_3), *m/z* (%), 612 [$M+NH_4^+$] (100), 595 [$M+H^+$] (6), 577 [$M+H^+-H_2O$] (13), 91 [Bn^+] (77), 593 [$M-H^+$] (2), 503 [$M-Bn^+$] (5); Anal. Calcd for $C_{38}H_{42}O_6$ (594.75); C, 76.74; H, 7.12. Found: C, 76.75; H, 7.08.

3.1.5. *rac*-(5aα,6α,7β,8α,9β,9aα)-6,7,8,9-Tetrakis(benzyloxy)-5a-[(2-methoxyethoxy)methoxy]decahydrobenzo[*b*]oxepin (10). 9 (397 mg, 0.67 mmol) was dissolved in 6 mL dry dimethylformamide. Sodium hydride (57 mg, 2.38 mmol) was added under vigorous stirring. After 45 min (0.130 mL, 1.15 mmol) 2-methoxy ethoxymethyl chloride was added and after 12 h additional sodium hydride (82 mg, 3.42 mmol) and 2-methoxy ethoxymethyl chloride was supplied. After 90 min the reaction mixture was quenched and dried in high vacuum. The residue was suspended in *tert*-butyl methyl ether and washed with phosphate buffer (pH 7) followed by brine. The organic layer was dried with Na_2SO_4 and filtered. The filtrate was dried under reduced pressure und the residue was chromatographed by preparative HPLC (RP-18, 88% MeOH, 40 mL/min, t_{Ret}=47.50 min) to give 393 mg of (0.58 mmol, 86% yield) **10** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42–7.21 (m, 20H, Ph), 5.27–5.04 (AB system, 2H, ^2J=6.8 Hz, OCH_2O), 5.02–4.55 (m, 8H, CH_2Ph), 4.16–3.62 (m, 2H, OCH_2–oxepane), 4.02 (dd, 1H, ^3J=9.5 Hz, H-7), 3.95 (dd, 1H, ^3J=9.4 Hz, H-9), 3.93–3.76 (m, 2H, OCH_2CH$_2$O), 3.62–3.54 (m, 2H, OCH$_2$CH_2O), 3.51 (dd, 1H, ^3J=9.2 Hz, H-8), 3.40 (s, 1H, OCH_3), 3.23 (d, 1H, ^3J=9.4 Hz, H-9a), 3.19 (d, 1H, ^3J=9.8 Hz, H-6), 2.52-2.36 (m, 1H, oxepane), 1.96–1.57 (m, 4H, oxepane), 1.52–1.42 (m, 1H, oxepane); ^{13}C NMR ($CDCl_3$) δ 139.50 (C), 139.31 (C), 139.02 (C), 138.79(C), 128.81–127.94 (CH, Bn), 91.53 (CH_2), 85.06 (CH), 84.38 (CH), 84.37 (CH), 83.38 (CH), 82.35 (CH), 82.06 (C), 76.64 (CH_2), 76.34 (CH_2), 76.32 (CH_2), 76.28 (CH_2), 72.33 (CH_2), 71.78 (CH_2), 67.63 (CH_2), 59.44 (CH_3), 34.00 (CH_2), 31.53 (CH_2), 21.07 (CH_2); DCI-MS (NH_3), *m/z* (%), 700 (100) [$M+NH_4^+$], 610 (19) [$M+NH_4^+-Bn^++H^+$], 593 (14) [$M+2H^+-Bn^+$], 91 (96) [Bn^+], 591 (14) [$M-Bn^+$], 501 (15) [$M-2Bn^++H^+$]; FAB-HRMS Calcd for $C_{35}H_{43}O_8$ [$M-Bn^+$]: 591.2958, found 591.2934.

3.1.6. *rac*-(5aα,6α,7β,8α,9β,9aα)-6,7,8,9-Tetrakis{[bis-(benzyloxy)phosphoryl]oxy}-5*a*-[(2-methoxyethoxy)methoxy]decahydrobenzo[*b*]oxepin (11). 10 (381 mg, 0.56 mmol) was dissolved in 5 mL ethanol and 0.05 mL di-*iso*-propylethylamine and palladium on charcoal (10% Pd, 379 mg, 0.36 mmol) was added under an argon atmosphere at −20°C. The argon atmosphere was exchanged to hydrogen in a self-built hydrogenation apparatus and the mixture was stirred for seven days at room temperature.

Finally, the hydrogen was exchanged to argon and the catalyst was removed by ultrafiltration. The filtrate was dried under reduced pressure. To the foamy residue 390 mg tetrazole (5.57 mmol) dissolved in 14 mL dry acetonitrile was added. Under argon, 1.85 mL (5.5 mmol) dibenzyl *N*,*N*-di-*iso*-propylphosphoamidite was added and the mixture was stirred for 18 h. The mixture was cooled to −40°C and 1.2 mL peracetic acid (32% wt, 5.76 mmol) were added. The solution was allowed to warm to room temperature and the volatile components were removed in high vacuum. The oily residue was purified by preparative HPLC (RP-18, 90% MeOH, 40 mL/min, t_{Ret}=31.40 min) to give **11** (426 mg, 56% yield) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42–7.09 (m, 40H, Ph), 5.20–4.79 (m, 20H, C*H*$_2$Ph, OC*H*$_2$O, H-7, H-9), 4.47 (ddd, 1H, ^3J=9.8 Hz=$^4J_{PH}$=9.8 Hz, H-8), 4.23 (dd, 1H, ^3J=10.2 Hz, H-6), 3.82–3.53 (m, 4H, OC*H*$_2$CH$_2$O, OC*H*$_2$–oxepane), 3.48–3.41 (m, 2H, OCH$_2$C*H*$_2$O), 3.32 (s, 3H, OC*H*$_3$),3.23 (d, 1H, ^3J=9.5 Hz, H-9a), 2.55–2.40 (m, 1H, oxepane), 1.91–1.87 (m, 5H, oxepane); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 136.88–135.95 (C, Bn), 129.26–127.93 (CH, Bn), 91.21 (CH_2), 81.13 (C), 80.99 (CH), 80.53 (CH), 78.47 (CH), 77.08 (CH), 75.63 (CH), 72.07 (CH_2), 71.84 (CH_2), 70.64–69.33 (CH_2, Bn), 67.49 (CH_2), 59.34 (CH_3), 33.92 (CH_2), 30.48 (CH_2), 21.22 (CH_2); ^{31}P NMR (81 MHz, $CDCl_3$) δ 1.00 (1P), 0.61 (1P), 0.33 (1P), −0.43 (1P); DCI-MS (NH_3), *m*/*z*(%), 1363 (3) [M+H$^+$], 91 (40) [Bn$^+$], 1271 (6) [M−Bn$^+$], 277 (36) [OP(OBn)$_2$O$^-$]; FAB-HRMS Calcd for $C_{63}H_{71}O_{20}P_4$ [M−Bn$^+$]: 1271.3489, found 1271.3455.

3.1.6. *rac*-(5aα,6α,7β,8α,9β,9aα)-)-5a-Hydroxy-6,7,8,9-tetrakis(phosphonooxy)decahydrobenzo[*b*]oxepin (3). **11** (52 mg, 38 μmol) was dissolved in 5 mL glacial acetic acid. Palladium on charcoal (10%, 136 mg, 128 μmol) were suspended. In a self-built hydrogenation apparatus the mixture was exposed to hydrogen under vigorous stirring for 18 h. The catalyst was removed by ultrafiltration, the filtrat was frozen and freeze-dried. The colorless residue was dissolved in distilled water and pourred on a cationion exchange resin (Dowex 50WX8, H$^+$ form). Acidic fractions were frozen and immediately freeze-dried to give **3** (19 mg, 35 μmol, 92% yield) as a colorless oil. ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.59–4.18 (m, 3H, H-7, H-8, H-9), 4.11 (dd, 1H, $^3J_{PH}$=^3J=9.9 Hz, H-6), 3.86–3.37 (m, 3H, OC*H*$_2$–oxepane, H-9a), 1.95–1.30 (m, 6H, oxepane); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 80.07 (CH), 79.74 (CH), 78.57 (CH), 78.45 (CH), 78.43 (CH), 75.49 (C), 69.98 (CH_2), 39.12 (CH_2), 30.22 (CH_2), 19.73 (CH_2); ^{31}P NMR (81 MHz, DMSO-d_6) δ 0.58 (2P), −0.18 (1P), −0.23 (1P); FAB-MS (glycerol), *m*/*z* (%), 555 [M+H$^+$] (7); FAB-MS (NBA), *m*/*z* (%), 553 [M−H$^+$] (95); FAB-HRMS Calcd for $C_{10}H_{21}O_{18}P_4$ [M−H$^+$] 552.9678, found 552.9676.

3.1.7. *rac*-(1*R*, 2*R*, 3*S*, 4*S*, 5*R*, 6*S*, 8*R*)-2,3,4,5-Tetrakis-(benzyloxy)-7,12-dioxatricyclo[6.3.1.0^{1,6}]dodecane (12). **8** (138 mg, 0.23 mmol) was dissolved in 10 mL aqueous ethanol (90%). Tris(triphenylphosphine)rhodium-(I)-chloride (46 mg, 0.43 mmol) and 0.04 mL di-*iso*-propyl-ethylamine (0.234 mmol) were added under argon. The mixture was heated to reflux in the dark for 18 h. Under reduced pressure the mixture was dried, the residue dissolved in *tert*-butyl methyl ether and washed with phosphate buffer pH 7 and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was dried and and the crude residue was prepurified by preparative HPLC (RP-18, 90% MeOH, 40 mL/min). The resulting mixture was dissolved in 5 mL chloroform, 0.1 mL trifluoroacetic acid was added, and the mixture was stirred for 3 days at room temperature. The solution was diluted with methylene chloride and extracted with phosphate buffer pH 7 and brine. The organic layer was dried over sodium sulfate and filtered. The filtrate was dried and and the crude residue was purified by preparative HPLC (RP-18, 90% MeOH, 40 mL/min, t_{Ret}=32.10 min). 74 mg **12** (0.13 mmol, 56% yield) was isolated as a colorless solid, mp 106–107°C. As a by-product 14 mg (26 μmol, 10% yield) **9** was isolated. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60–7.25 (m, 20H, Ph), 5.73 (br s, 1H, OC*H*O), 5.16–4.75 (m, 8H, OC*H*$_2$Ph), 4.18 (d, 1H, ^3J=6.1 Hz, H-6), 4.00 (dd, 1H, ^3J=9.5 Hz, H-3), 3.71 (dd, 1H, ^3J=9.8 Hz, 6.2 Hz, H-5), 3.53 (dd, 1H, ^3J=9.5 Hz, H-4), 3.52 (d, 1H, ^3J=9.7 Hz, H-2), 2.36–1.64 (m, 5H, thp-ring), 1.40–1.22 (m, 1H, thp-ring); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ=139.14 (C), 139.14 (C), 139.01 (C), 138.41 (C), 129.34–127.85 (CH, Bn), 103.87 (CH), 86.08 (CH), 83.20 (CH), 83.10 (C), 82.53 (CH), 81.72 (CH), 81.67 (CH), 76.90 (CH_2), 76.18 (CH_2), 76.00 (CH_2), 74.62 (CH_2), 31.27 (CH_2), 30.60 (CH_2), 16.59 (CH_2); DCI-MS (NH_3), *m*/*z* (%), 591 [M−H$^+$] (85), 501 [M−Bn$^+$] (88), 411 [M−2Bn$^+$+H$^+$] (15), 321 [M−3Bn$^+$+2H$^+$] (3), 610 [M+NH$_4^+$] (58), 593 [M+H$^+$] (1), 520 [M+NH$_4^+$−Bn] (18); Anal. Calcd for $C_{38}H_{40}O_6$ (592.73); C, 77.00; H, 6.80. Found: C, 77.19; H, 6.69.

3.1.8. *rac*-(1*S*, 2*R*, 3*S*, 4*R*, 5*S*, 6*S*, 8*R*)-2,3,4,5-Tetrakis-{[bis(benzyloxy)phosphoryl]oxy}-7,12-dioxatricyclo-[6.3.1.0^{1,6}]dodecane (13). **12** (0.159 g, 0.267 mmol) was dissolved in 5.5 mL ethanol at −20°C. A suspension of 0.222 g palladium on charcoal (10% Pd, 0.209 mmol) in 5.5 mL ethanol under an argon atmosphere at −20°C was added. The argon atmosphere was exchanged to hydrogen in a self-built hydrogenation apparatus and the mixture was stirred for eight days at room temperature. Finally, the hydrogen was exchanged to argon and the catalyst was removed by ultrafiltration. The filtrate was dried in high vacuum and the residue was dissolved in dry acetonitrile. Then tetrazole (160 mg, 2.286 mmol) and dibenzyl *N*,*N*-di-*iso*-propylphosphoramidite (0.705 ml, 2.098 mmol) were added under argon and the mixture was stirred for 18 h. After cooling to −40°C, peracetic acid (32% in acetic acid, 0.44 mL, 2.1 mmol) was added. The solution was allowed to warm to room temperature and all volatile components were removed in high vacuum. The oily residue was chromatographed by preparative HPLC (88% MeOH, 40 mL/min, t_{Ret}=38.45 min) to give 173 mg (0.20 mmol, 59% yield from **12**) **13** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45–7.17 (m, 40H, Ph), 5.50 (br s, 1H, OC*H*O), 5.19–4.77 (m, 20H, C*H*$_2$Ph, H-2, H-3, H-4, H-5), 4.18 (d, 1H, ^3J=4.7 Hz, H-6), 2.12-1.90 (m, 1H, thp-ring), 1.82–1.45 (m, 5H, thp-ring); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 136.18 (C), 136.17 (C), 136.17 (C), 136.16 (C), 129.17–128.20 (CH, Bn),104.23 (CH), 81.22 (C), 78.77 (CH), 78.33 (CH), 78.20 (CH), 77.76 (CH), 77.31 (CH), 70.44–68.77 (CH_2, Bn), 31.33 (CH_2), 30.22 (CH_2), 16.17 (CH_2); ^{31}P NMR (81 MHz, $CDCl_3$) δ −0.16 (1P), −0.35 (1P), −0.95 (2P); DCI-MS (NH_3), *m*/*z* (%), 1271 [M−H$^+$]

(4), 1181 [M−Bn^+] (100), 1091, [M−2Bn^++H^+] (32), 1290 [M+NH_4^+] (3), 1272 [M+H^+] (1); DCI-HRMS Calcd for $C_{59}H_{61}O_{18}P_4$ [M−Bn^+]:1181.2809, found 1181.2819.

3.1.9. *rac*-(1*S*, 2*R*, 3*S*, 4*R*, 5*S*, 6*S*, 8*R*)-2,3,4,5-Tetrakis-(phosphonooxy)-7,12-dioxatricyclo[6.3.1.0^{1,6}]dodecane (4). Under an argon atmosphere (168 mg, 0.13 mmol) **13** was dissolved in 3 mL ethanol cooled to −20°C. A suspension of 190 mg palladium on charcoal (10% Pd, 0.179 mmol) in 5 mL ethanol under an argon atmosphere at −20°C was added. The argon atmosphere was exchanged to hydrogen in a self-built hydrogenation apparatus and the mixture was stirred for seven days at room temperature. Finally, the hydrogen was exchanged to argon and the catalyst was removed by ultrafiltration. The filtrate was dried in high vacuum to give 60 mg (0.11 mmol, 82% yield) of **4** as the free acid. 33 mg were treated with Dowex 50 WX8, sodium form to give 17 mg (23 μmol, 17% yield) **4** as the sodium salt. ^{1}H NMR (200 MHz, D_2O) δ 5.63 (br s, 1H, OC*H*O), 4.32–3.91 (m, 5H, H-2, H-3, H-4, H-5, H-6), 2.12–1.90 (m, 1H, thp-ring), 1.85–1.32 (m, 5H, thp-ring); ^{13}C NMR (50.3 MHz, D_2O) δ 103.82 (CH_2), 81.06 (CH), 80.69 (CH), 80.06 (CH), 77.75 (CH), 76.46 (CH), 30.30 (CH_2), 29.57 (CH_2), 15.46 (CH_2); ^{31}P NMR (81 MHz, D_2O) δ 2.13 (1P), 1.85 (1P), 0.95 (2P); FAB-MS (glycerol), *m*/*z* (%), 573 [M−2H^++Na^+] (13), 551 [M−H^+] (30); FAB-HRMS Calcd for $C_{10}H_{19}O_{18}P_4$ [M−H^+] 550.9522, found 550.9520.

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