Stereoselective Synthesis of Spiroacetal Domain Derivatives of the Plant Glycoside Ranuncoside and of Okadaic Acid and Dinophysistoxins-1 and 2 From Marine Algae

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Abstract

Spiroacetals constitute the central structural core element of numerous natural products and are mostly represented as bicyclic or tricyclic domains. Typical natural products with tricyclic spiroacetals are (+)-ranuncoside, a glycoside isolated from plants of the Ranunculaceae family, and the algal toxins (+)-okadaic acid and the (+)-dinophysistoxins-1 and 2. These substances possess a spiro furan-dioxane-pyran ring system and a spiro furan-pyran-pyran scaffold, which are both essential for biological activity. The corresponding analogs with spiro furan-dioxane-cyclohexane framework have so far neither been found in living organisms nor been synthesized. To close this gap and to generate candidates for structure-activity relationship studies which could lead to the discovery of novel antibiotics and selective anticancer agents, we have developed an efficient and stereocontrolled synthetic route to analogous domains of the above natural products. Pyran-dioxane-cyclohexane tricycles were used as starting materials and, via ring contraction, yielded the 2 derivatives with spiro (R)- and spiro (S)-configuration and tricyclic ring system and (ent)-7, respectively. The stereochemistry and conformation of all novel products were solved by nuclear magnetic resonance spectroscopy.

Keywords

spiroacetal, plant glycoside, marine toxin, antibiotic, anticancer agent

Received: September 23rd, 2020; Accepted: October 13th, 2020.

A multitude of natural products of insect, marine, bacterial, or plant origin is characterized by spiroacetal domains as a central structural core element. Generally, these central domains are represented as bicyclic and tricyclic spiroacetal ring systems which possess complex stereochemistry and specific biological activities. While investigating natural bioactive phytoagents, Tscheche et al. described that the 2 toxic plants Ranunculus repens and Helleborus foetidus contain glycosides with lactone forming aglycon which displays considerable antibiotic properties. They extracted a novel crystalline compound with positive rotation from a pool of active substances and named it ranuncoside due to its origin from Ranunculaceae species. The same group elucidated its structure by peracetylation and subsequent analysis of its 90 MHz 1H NMR spectrum. (+)-Ranuncoside (Figure 1) is a spiro furan-dioxane-pyran tricycle with a complex substitution pattern and stereochemistry. The central dioxane ring is fused 2-fold with the pyranose moiety in trans-manner and is additionally linked to a saturated γ-lactone ring in spiroacetal manner. Mariezcurrena et al. determined the X-ray structure of 1, thereby establishing its stereochemistry at the spiro carbon. A subsequent full paper was published by the same group, revealing further structural and quantitative details such as bond lengths and a molecule of water, hydrogen bonded to the oxygen atom of the exocyclic CH2OH-group of the pyranose moiety. Martinek reported the isolation of the crystalline monohydrate of (+)-ranuncoside from dried stems, leaves, and flowers of Helleborus niger.

The biosynthesis of 1 in Helleborus foetidus was described by Tscheche et al. by in vivo incorporation experiments with radioactively labeled 5-hydroxy[1,14C] and [4,14C] levulinic acid. Biochemical tests of the antibiotic potential of (+)-ranuncoside have, to our knowledge, not yet been published.

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Confusingly, the name ranuncoside was later also used for two completely different structures: (1) a pentacyclic triterpenoid glycoside from *Hydrocotyle ranuncoloides* (Apiaceae) and (2) an olefinic glycoside from *Ranunculus muricatus* (Ranunculaceae) with unsolved stereochemistry in the whole hexose moiety.

(+)-Okadaic acid and (+)-dinophysistoxin-1 and 2, formula 3 and 4, possess a higher molecular weight and a more complex molecular structure, as compared with (+)-ranuncoside 1 (Figure 1). The main characteristic is the central tricyclic spiro furan-pyran-pyran framework (segment B) which is C3-bridged on both sides with 2 additional bicyclic spiroacetals. Natural products 2, 3, and 4 are algal toxins that accumulate in shellfish and they are potent serine/threonine phosphatase (ser/thr PP) inhibitors, causing diarrhea and gastrointestinal symptoms. Two independent total syntheses of (+)-okadaic acid 2 were published by the Forsyth and Ley groups in 1997 and 1998, respectively. Ten years later, the synthesis of the terminal eastern bicyclic spiroacetal part of dinophysistoxin-2 (structure 4) was reported by Forsyth and Wang. The unique physiological features of the okadaic acid class of tumor promoters, as well as the mechanism of action of these cell death-inducing microbial protein phosphatase inhibitors have also been reviewed.

It is interesting to note that terrestrial plants and algae produce different tricyclic spiro ring systems. While (+)-ranuncoside 1 contains the spiro furan-dioxane-pyran domain with 4 oxygen atoms in total, the marine algal toxins 2, 3, and 4 instead possess the spiro furan-pyran-pyran motif with only 3 oxygen atoms. The structural analogous domains with the spiro furan-dioxane-cyclohexane ring systems and 3 oxygen atoms, structure 7 and (ent)-7, have not been found in nature until now (Figure 2).

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**Figure 1.** The plant glycoside (+)-ranuncoside 1, the marine algal toxins (+)-okadaic acid 2, and the (+)-dinophysistoxins-1 and 2 (structures 3 and 4) are characterized by a tricyclic spiroacetal domain (highlighted in red).

**Figure 2.** Structures and stereochemistries of the tricyclic spiroacetal domain of (+)-ranuncoside 1 (structure 5), of the segment B of (+)-okadaic acid 2 and the (+)-dinophysistoxins 3 and 4 (structure 6). The corresponding structural analogous domains, 7 and (ent)-7, differ from 5 and 6 by loss or movement of 1 oxygen atom.
Due to the remarkable bioactivity of numerous natural compounds with spiroacetal domains, we developed the synthesis of novel bicyclic spiroacetal motifs by highly stereoselective spiroacetalization of pyranodioxanes. Here, we adapted this methodology to linear fused pyran-dioxane-cyclohexane tricycles in order to obtain tricyclic spiroacetal domains, since we considered it to be the most logical and feasible approach. Of the derivatives we aimed to synthesize by this methodology, particularly those with the spiro domain 7 and (ent)-7 were considered to be valuable tools for structure-activity relationship studies and for the development of novel and effective antibiotics and anticancer agents.

Results and Discussion

The chemical elaboration of derivatives with spiro furan-dioxane-cyclohexane ring system 7 and (ent)-7 is difficult, and, to our knowledge, no approach is yet known. Mondon et al. described a bis-half acetal with structure 10, in a study investigating members of the Cneoraceae plant family. They obtained 10 by chemical derivatization of cneorine C, a bitter substance in the leaves of *Cneorum pulverulentum* (synonym: *Neochamaelea pulverulenta*), a flowering plant in the family Rutaceae, native to the Canary Islands. Osmium tetroxide hydroxylation of 8 to the cis-diol 9 and subsequent sodium periodate oxidative cleavage under neutral reaction conditions gave 10 in crystalline form. Compound 10 has only little structural resemblance to the spiro furan-dioxane-cyclohexane framework 7, due to (1) its cyclized bis-half acetal structure and (2) its unknown stereochemistry of the 2 annulation positions of dioxane and the cyclohexane ring (Scheme 1).

Our stereoselective synthesis of derivatives of the spiroacetal 7, viz, the domain of (+)-ranuncoside 1 and of the spiroacetal (ent)-7, the domain of (+)-okadaic acid 2, and the (+)-dinophysistoxins-1 and 2 (structures 3 and 4), described here, started from D-glucose-derived linear fused pyran-dioxane-cyclohexane tricycles.

**Synthesis of Pyran-dioxane-cyclohexane Tricycles 12, 17, and 25**

In 2004, we published the synthesis of pyran-dioxane bicycles through bisacetal annulation of 2-ketosugars to glycol. In a directly connected paper, we described the glycosylation of (R,R)-configured and (S,S)-configured cyclohexane-1,2-diols with ulosyl bromide. Here, we describe an established 50 g synthesis of 11 from D-glucose (see the Experimental section). Reaction of 11 with (R,R)-cyclohexanediol in dimethyl formamide furnished the hitherto unknown linear fused pyran-dioxane-cyclohexane tricycle 12 in 71% yield (Scheme 2). The bisacetal annulation occurred via mono-O-α-glycosylation along with cis-ketalization at the C-2 ulose carbonyl group of 11 (α-α′-addition). The 2 newly formed stereogenic centers of the central dioxane ring have 4αR and 10αS configuration. Application of silver triflare as soluble promoter and tetrahydrofuran or dichloromethane as solvent gave mixtures of 12 and 17 (Table 1).

The isomeric pyran-dioxane-cyclohexane tricycle 17, which has a reversed 4α′ and 10αR configuration, was obtained in 87% yield by the reaction of 11 with (R,R)-cyclohexanediol in dichloromethane and in the presence of silver carbonate as promoter and acid scavenger (β-α′-addition). The reaction was faster in tetrahydrofuran, but the yield was slightly lower (Table 1).

In contrast to the 2 tricycles above, the isomer 25 possesses an (S,S)-cyclohexane aglycon. The 4α′ and 10αR configuration originates from the β-α′-addition of (S,S)-cyclohexanediol to ulosyl bromide 11.

**Synthetic Access to the Spiroacetal Domain Derivative of (+)-Ranuncoside 1**

Ring contraction of 12 with benzoic acid anhydride in the presence of perchloric acid (70%) gave the spiroacetal 16 in 94% yield. The reaction started by protonation of 12 to 13, which undergoes simultaneous ring contraction and water elimination.

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**Scheme 1.** Hydroxylation of cneorine C (8) with osmium tetroxid to the cis-diol 9 followed by sodium periodate cleavage to the bis-half acetal 10 with unknown stereochemistry of the 2 hydroxyl groups. For clarity, the reacting double bond and the hydroxyl groups are highlighted in blue, while framework 7 is highlighted in red.
Scheme 2. α-cis- or β-cis-Addition of (R,R)-cyclohexanediol on ulosylbromide 11 gave the pyran-dioxanecyclohexane tricycles 12 and 17. Thus, protonation of 12 (structure 13), ring contraction (structure 14), water addition (structure 15), and final benzoylation yielded 16. Based on 172 as starting material, the reaction sequence was identical and differed only by the primary epimerization of 17 to 19. Ring contraction of 17 did not proceed due to the lack of stabilizing anomeric effects in the final product 24 (Figure 3). For clarity, the resulting tricyclic spiroactal domain is highlighted in red, and the configurational transcriptors of the dioxane carbons are shown in blue.
to the oxonium carbenium ion 14 (Scheme 2). The expected migration of the bond 10-10a, which is antiperiplanar to the departing hydroxyl group at C4a (structure 12 in Figure 3) does not proceed because compounds with 1,3-dioxolane structure could not be detected. The reason is probably the instability of the expected product along this pathway under the applied drastic acidic reaction conditions. In 1 further equilibrium reaction, a molecule of water attacks 14 from the less hindered underside of the molecule, forming the half acetal 15. This intermediate is immediately trapped by benzoylation to furnish the spiroacetal 16. Driving force of the highly selective esterification is the comparably even more reactive secondary hydroxyl group of 15, compared with the unreactive tertiary hydroxyl group of the starting material 12. The reaction is highly stereoselective, probably stereospecific, because no side-products were found at all. The chair conformation of 16 illustrates the stabilization of 2 anomic effects (Figure 3). Compound 16 has the exact same stereochemistry as in framework 7, which corresponds to structure 5, the spiroketal domain of (+)-ranuncoside 1.

Application of the same reaction conditions to the diastereomeric linear fused tricycle 1722 (Scheme 2) also gave the spiroacetal 16 (70% yield). The reaction sequence is identical and differs only by the primary epimerization of 17 to 19 (via the open chain form 18) followed by protonation to 20 and ring contraction to 14.

In summary, both linear fused tricycles with (R,R)-cyclohexane portion, that is, compounds 12 and 17, are suitable starting materials for the construction of derivatives of (+)-ranuncoside 1.

Table 1. Glycosylation of (R,R)-Cyclohexanediol With Ulosylbromide 11: Reaction Conditions, Ratio of Product 12 and 17, as well as Overall Yield.

| Promoter | Solvents | Time (h) | Temperature (°C) | Ratio 12:17 | Yield (%) |
|----------|----------|----------|------------------|-------------|-----------|
| -        | DMF      | 72.0     | 20               | 1:0         | 71        |
| AgOTf    | THF      | 2.0      | 0                | 1:1         | 83        |
| AgOTf    | CH₂Cl₂   | 0.75     | 0                | 3:7         | 80        |
| Ag₂CO₃   | CH₂Cl₂   | 2.0      | 40               | 0:1         | 8722      |
| Ag₂CO₃   | THF      | 0.5      | 65               | 0:1         | 75        |

Abbreviations: Ag₂CO₃, silver carbonate; AgOTf, silver triflate; CH₂Cl₂, dichloromethane; DMF, dimethylformamide; THF, tetrahydrofuran.

* Determined by 1H-nuclear magnetic resonance.

b Overall yield.

Figure 3. All-chair conformations of (a) the spiroacetal derivatives 16, 24, and 29, indication the anomic effects by blue arrows and (b) of the starting material 12, illustrating the operative nuclear Overhauser effect proven by 1H-nuclear magnetic resonance spectroscopy.
Synthetic Access to the Spiroacetal Domain Derivative of (+)-Okadaic Acid 2 and the (+)-Dinophysistoxins-1 and 2 (3 and 4)

Reaction of the linear fused pyran-dioxane-cyclohexane tricycle 25,22 possessing a (S,S)-cyclohexane aglycon and 4aS and 10aR configuration, with benzoic acid anhydride and in the presence of perchloric acid (70%) gave the spiroketal 29 in 95% yield (Scheme 3).

As before, the reaction sequence started by protonation (structure 26), followed by simultaneous ring contraction and elimination of water (structure 27). Addition of water (structure 28) and benzoylation gave the final product 29, which has 2 stabilizing anomeric effects (Figure 3) and the ring framework of ent-7 (marked in red).

This represents the isomer of motif 6, which is also found in the spiroketal domain (segment B) of (+)-okadaic acid 2 and the (+)-dinophysistoxins 3 and 4.

The 2 synthesized spiroacetals 16 and 29 are deemed suitable starting materials/structures for the construction of lead structures for the design of novel drugs such as antibiotics and tumor promoters.

Structural Elucidation

Structure and all-chair conformation of the synthesized starting material, the pyran-dioxane-cyclohexane tricycle 12 (Figure 3) was fully resolved by 500 MHz 1H and 13C NMR analysis (see Experimental section). The connection of pyran and dioxane ring, viz, the stereochemistry of the newly formed stereogenic centers at C-4a and C-10a, was revealed by the presence of the 2 nuclear Overhauser effects (NOEs) between 4H ↔ 5a-H and 9a-H ↔ 10a-H. The configuration and conformation of the 2 other starting materials 17 and 25 are well documented.22

The final products, the spiro furan-dioxane-cyclohexane tricycles 16 and 29, have inverted rotations ([α]D 20 = +51.7 and [α]D 20 = -100.9), respectively, and are furthermore characterized by reversed configuration in the dioxane and cyclohexane part (2R, 3R, 4aR, 8aR vs 2S, 3S, 4aS, 8aS) (Figure 3). The spectra are very similar, and as expected, the shifts of the protons of the 4 CH2-groups of the cyclohexane portion are found as a broad multiplet in the range of δ 1.1-2.1 ppm. The resonances for the 2 protons, 4a-H and 8a-H, at the dioxane and cyclohexane linkage position are in each case a multiplet around δ 3.8 and 4.0 ppm. The shifts H A and H B of the exocyclic CH2OBz-group are partly overlapped double doublets around δ 4.7 and 4.8 ppm. The sharp singulet at δ 6.45 (for 16) and 6.33 ppm (for 29) is assigned to the dioxane 3H because of the loss of neighborly protons.

The furan chemical shifts and coupling constants, 3ʹ-H, 4ʹ-H, 5ʹ-H, as well as of the spiro C-2 resonance of spiroacetals 16 and 29 are summarized in Table 2. The values are in accordance with the trans-configuration of the substituents. The pronounced shift difference of 0.35 ppm for 4ʹ-H (5.53 vs 5.88

| Compounds | 3ʹ-H | 4ʹ-H | 5ʹ-H | J3ʹ,4ʹ | J4ʹ,5ʹ | Spiro C-2 |
|-----------|------|------|------|--------|--------|----------|
| 16a       | 5.79ps | 5.53pd | 4.62dd | <0.3  | 4.2  | 103.8 |
| 29a       | 5.96d  | 5.88dd | 4.64dd | 6.9   | 5.3  | 100.2  |

Abbreviation: NMR, nuclear magnetic resonance.

*The complete data are listed in the Experimental section.
ppm) and the small value of the coupling constant of \( J_{\gamma', \gamma''} = <0.3 \text{ Hz} \) for 16 compared to \( J_{\gamma', \gamma''} = 6.9 \text{ Hz} \) for 29 substantiate that they have different envelope conformations. The same result was also found and discussed in detail by Lemieux and Nagarajan\(^{23} \) for the hexaacetate of difructose anhydride 1, in which 2 furanose conformations are present.

**Conclusions**

The plant glycoside (+)-ranucoside 1 and the algal toxins (+)-okadaic acid 2, and (+)-dinophysistoxin-1 and 2 (3 and 4) are characterized by pronounced biological activity and complex stereochemistry. Their central core elements are 2 different spiroacetal domains, the tricyclic spiro furan-dioxane-pyran ring system 5, and the spiro furan-pyran-pyran framework 6. After having developed stereoselective syntheses of novel bicyclic spiroacetal motifs of numerous natural products,\(^{20} \) we here describe the synthesis of appropriate compounds possessing the tricyclic spiro domains 7 and (ent)-7.

The 2 new spiroacetal domain derivatives of ranuncoside, and of okadaic acid and dinophysistoxin-1 and 2, the compounds 16 and 29, were synthesized by ring contraction of the linear fused pyran-dioxane-cyclohexane tricycles 12, 17,\(^{25} \) and 25\(^{25} \) with benzoic acid anhydride in the presence of perchloric acid. The latter 3 are accessible by the reaction of (R,R)- and (S,S)-cyclohexane diol with ulosylbromide 11, which could be prepared by a well-proven 4-step synthesis from D-glucose.

The structure of the new compounds, the starting material 12 and the final products 16 and 29, could be resolved by \(^1\)H and \(^{13}\)C NMR analysis. Thus, in 12, the connection of pyran and dioxane ring, viz, the stereochemistry of the newly formed stereogenic centers at C-4a and C-10a became clear by the presence of the 2 NOEs between 4H ↔ 5a-H and 9a-H ↔ 10a-H (Figure 3). The final products, 16 and 29, have inverted rotations and reversed configuration in the central ring. Their \(^1\)H and \(^{13}\)C spectra are very similar and the chemical shifts could be assigned without doubt. The pronounced differences in the shift value and coupling constant for the furan \( 4'-\text{H} \) is in accordance with the results of Lemieux and Nagarajan.\(^{23} \) The synthesized compounds were considered to be valuable tools for future structure-activity relationship studies and for the development of novel and effective antibiotics and antineoplastic agents.

**Experimental**

Thin-layer chromatography (TLC) was performed on POLYGRAM SILG/UV\(_{254} \) (Macherey Nagel & Co.). Preparative chromatographic separations were carried out on columns with Merck silica gel 60 (15-40 µm) and Merck precoated silica gel plates 60 F\(_{254} \), 20 × 20 cm, 0.25 mm. Melting points were determined on a Bock-Monoskop VS or on a Büchi SMP-20 and are uncorrected. Specific optical rotations were determined on a Perkin-Elmer Polarimeter 241 in 1 dm cuvettes at a wavelength of 589 nm. NMR spectra were measured on a Bruker WM 300 and DRX 500 spectrometer at 303 K using trimethylsilane as internal reference or by calibration with the shift of the solvent of the sample.\(^{26} \) The abbreviation of the multiplicities of the shifts are indicated as s for singlet, d for doublet, t for triplet, q for quartet, sext for sexet, oct for octet, m for multiplet, br for broad, and p for pseudo. Mass spectra were run on a MAT 311 mass spectrometer (Varian). Elemental analyses were performed on a Perkin Elmer 240 Elementar Analyser.

\((+)-(2R,3\text{R},4\text{S},4\text{aR},5\text{aR},9\text{aR},10\text{aS})-3,4\text{-Bis-Benzoyloxy}-2\text{-}\text{(Benzoyloxy)methyl-4a-Hydroxy-Decahydro-2H-Pyrano}[2,3-b][1,4]\text{benzodioxin} \) (12)

Dimethylformamide without a silver salt promoter. A mixture of ulosylbromide 11 (553 mg, 1.00 mmol), \((1\text{R,2R})\text{-trans-1,2-cyclohexanediol} \) (116 mg, 1.00 mmol) and of freshly annealed molecular sieve 4 Å (2 g) were stirred in anhydrous dimethylformamide (10 mL) for 3 days at room temperature. The reaction mixture was chromatographed twice on a silica gel column (10 × 2 cm, toluene/ethyl acetate 4:1) and yielded 420 mg (71%) of 11 with \( [\alpha]_{20}^\text{D} = 0.52 \) (toluene/ethyl acetate 4:1). An analytical sample was obtained by preparative chromatography on a silica gel plate (n-hexane/diethyl ether 2:1) and gave an amorphous powder with \( [\alpha]_{20}^\text{D} = +42.9 \) (\( c = 1.0, \text{CHCl}_3 \)).
Silver carbonate in tetrahydrofuran. A mixture of (1R,2R)-trans-1,2-cyclohexanediol (116 mg, 1.00 mmol) and silver carbonate (276 mg, 1.00 mmol) in anhydrous tetrahydrofuran (10 mL) was stirred in the presence of a freshly annealed molecular sieve 4 Å (2 g) at room temperature for 15 minutes. Ulosylbromide 11 (553 mg, 1.00 mmol) was then added and stirred continuously for 30 minutes at 65 °C in the dark. Filtration through a pad of kieselguhr and evaporation of the filtrate in vacuo left a syrup which was purified by flash chromatography on a silica gel column (4 × 2 cm, n-hexane/diethylether 1:1): 443 mg (75%) 17 with identical physical and spectroscopic data as described by Lichtenthaler and Cuny.22

Silver carbonate in dichloromethane. A mixture of (1R,2R)-trans-1,2-cyclohexanediol (116 mg, 1.00 mmol) and silver carbonate (276 mg, 1.00 mmol) in dichloromethane (200 mL) and washed consecutively with saturated sodium bicarbonate (NaHCO₃) solution (50 mL) and water (2 × 50 mL). Drying (sodium sulfate [Na₂SO₄]) and evaporation to dryness in vacuo left a syrup which was purified on a short silica gel column (4 × 2 cm, n-hexane/diethyl ether 20:1 → 4:1): 490 mg (83%) of an approximate 1:1 mixture of 12 and 17 (1H-NMR).

Silver triflate in tetrahydrofuran. A solution of (1R,2R)-trans-1,2-cyclohexanediol (116 mg, 1.00 mmol) in anhydrous tetrahydrofuran (10 mL) was cooled down to 0 °C. The solutions of ulosylbromide 11 (553 mg, 1.00 mmol) in tetrahydrofuran (1 mL) and silver triflate (257 mg, 1.00 mmol) in tetrahydrofuran (1 mL) were successively added with a syringe and stirred for 2 hours at 0 °C in the dark. The ice bath was removed and stirring continued for another 1 hour at room temperature. The mixture was then diluted with dichloromethane (200 mL) and washed consecutively with saturated sodium bicarbonate (NaHCO₃) solution (50 mL) and water (2 × 50 mL). Drying (sodium sulfate [Na₂SO₄]) and evaporation to dryness in vacuo left a syrup which was purified on a short silica gel column (4 × 2 cm, n-hexane/diethyl ether 20:1 → 4:1): 490 mg (83%) of an approximate 1:1 mixture of 12 and 17 (1H-NMR).

(+)-(2R, 3R, 3'S, 4R, 4'S, 5'R, 5'S, 8aR)-3,3'-Tris-Benzoyloxy-5'-(Benzoyloxy)methyl-Octahydro-3H,3'H-Spiro[benzo[b]/1,4]dioxine-2,2'-Furan (16)

Starting material 12. Benzonic acid anhydride (2.26 g, 10.00 mmol) and aqueous perchloric acid (70%, 50 µL, 0.58 mmol) were added to a stirred solution of 12 (0.59 g, 1.00 mmol) in dichloromethane (50 mL) and stirred for 10 minutes at room temperature. The mixture was then diluted with dichloromethane (200 mL) and washed consecutively with water (50 mL), saturated NaHCO₃ solution (3 × 50 mL), and water (50 mL). Drying (Na₂SO₄) and evaporation to dryness in vacuo left a syrup which was purified on a silica gel column (10 × 2 cm, n-hexane/diethyl ether 0.1:1 → 1:1): 1.1 g (84%).

(+)-(2R, 3R, 3'S, 4R, 4'S, 5'R, 5'S, 8aR)-3,3'-Tris-Benzoyloxy-5'-(Benzoyloxy)methyl-Octahydro-3H,3'H-Spiro[benzo[b]/1,4]dioxine-2,2'-Furan (16)
and evaporation to dryness in vacuo left a syrup which was dissolved in methanol (600 mL) and heated for 30 minutes to reflux. After cooling down to room temperature, the crystalline product was filtered off by suction: d-glucose pentabenzoxoate consisted of a 4:1-mixture of α/β-anomers, 132 g (94%), m.p. 185 °C; literature25 95%, m.p. 187 °C.

1H NMR (500 MHz, CDCl 3) δ 4.52 (dd, 1H, 6-H), 4.69 (dd, 1H, 6-H), 5.86 (pt, 1H, 4H), 5.89 (pt, 1H, 2H hidden by 4H of α-anomer), 6.08 (pt, 1H, 3H), 6.34 (d, 1H, 7H), 7.25-8.18 (m, 25H, 5-C 6H5CO); J1,2 = 8.0, J2,3 = 9.5, J3,4 = 9.5, J5,6 = 9.8, J5,6A = 4.8, J5,6B = 3.0, J6,7gem = 12.4 Hz.

13C NMR (125.8 MHz, CDCl 3) δ 62.62 (C-6), 69.01 (C-4), 70.58*, 70.62*, 70.64* (C-2, C-3, C-5), 90.18 (C-1), 128.1-130.4 (aromatic meta and para-C), 133.1-134.2 (aromatic ortho-C), 164.50, 165.25, 165.46, 166.02, 166.18 (5 C 6H5CO); *shifts could be interchanged.

Minor β-anomer: δ 4.44 (dd, 1H, 5H), 4.54 (dd, 1H, 6-H), 4.69 (dd, 1H, 6-H), 5.86 (pt, 1H, 4H), 5.89 (pt, 1H, 2H hidden by 4H of α-anomer), 6.08 (pt, 1H, 3H), 6.34 (d, 1H, 7H), 7.25-8.18 (m, 25H, 5-C 6H5CO); J1,2 = 8.0, J2,3 = 9.5, J3,4 = 9.5, J5,6 = 9.8, J5,6A = 4.8, J5,6B = 3.0, J6,7gem = 12.4 Hz. 13C NMR (125.8 MHz, CDCl 3) δ 62.83 (C-6), 69.25 (C-4), 71.02* (C-2), 72.98 (C-3), 73.63 (C-5), 92.85 (C-1), 128.1-130.4 (aromatic meta and para-C overlapped of α-anomer-C), 133.1-134.2 (aromatic ortho-C overlapped of α-anomer-C), 164.2-166.4 (5 C 6H5CO overlapped of α-anomer-C).

In a 3-necked round-bottom flask with a mechanical stirrer and dropping funnel, hydrobromic acid (HBr) 1.2 M solution in glacial acetic acid (80 mL, 0.5 mol) was added under stirring at room temperature to a solution of 1,2,3,4,6-penta-O-benzoyl-d-glucopyranosyl bromide 31. After stirring for another 2 hours, the mixture was diluted with dichloromethane (100 mL) and washed consecutively with iced water (3 × 50 mL), saturated sodium bicarbonate (NaHCO3) solution (100 mL) and water (3 × 50 mL). Drying (Na2SO4) and evaporation to dryness in vacuo left a brown syrup which was used without further purification: 65 g (70%); literature23 ~90%.

2,3,4,6-Tetra-O-Benzyl-5-Anhydro-1-d-Arabino-Hex-1-Enitol (Tetra-O-Benzyl-2-Hydroxy-d-Glucal) (32). In a 3-necked round-bottom flask with a mechanical stirrer, 33.2 g (0.05 mol) of the above prepared 2,3,4,6-tetra-O-benzoyl-d-glucopyranosyl bromide 31 were dissolved in anhydrous acetone (70 mL). Sodium iodide (NaI) (8.2 g, 0.055 mol) was added and the mixture was stirred for 30 minutes at room temperature. Afterward, diethyl amine 35 mL (0.5 mol) was added and the mixture stirred for another 2 hours. The mixture was diluted with chloroform (50 mL) and washed consecutively with water (50 mL) and 2 N HCl until the solution became acidic (pH 5-6). Drying (sodium sulfate [Na2SO4]) and evaporation to dryness in vacuo left a syrup which was
crystallized from methanol (200 mL): 22.1 g (76%), m.p. 119 °C-120 °C; literature 23 90%, m.p. 121 °C-122 °C.

1H NMR (500 MHz, CDCl₃) δ 4.73 (dd, 1H, 6-H₃), 4.90 (dd, 1H, 5H, overlapped with 6-H₄), 4.91 (dd, 1H, 6-H₅; overlapped with 5H), 5.85 (pt, 1H, 4H), 6.12 (d, 1H, 3H), 6.99 (s, 1H, 1H), aromatic meta-H: 7.35-7.44 (m, 6H), and 7.47 (pt, 2H), aromatic para-H: 7.50-7.62 (m, 4H), aromatic ortho-H: 7.98 (dd, 2H), 8.03 (m, 4H), and 8.12 (dd, 1H); J₆,₈ = 3.7, J₄,₅ = 4.0, J₅,₆ = 3.5, J₆,₅b = 7.2, J₂,₆em = 10.8, aromatic Jortho-meta = 8.3, Jortho-para = 1.1, Jmeta-para = 8.0 Hz.

13C NMR (125.8 MHz, CDCl₃) δ 61.66 (C-6), 66.73 (C-3), 68.37 (C-4), 73.97 (C-5), 128.53, 128.59 (2s), 128.67 (aromatic meta-C), 129.85, 129.94, 130.20 (2s) (aromatic para-C), 133.34, 133.50, 133.64, 133.74 (aromatic ortho-C), 139.88 (C-1), 161.15, 165.47, 165.61, 166.18 (C₆H₅CO).

Acknowledgment
The author thanks Prof. Dr. M. Reggeli for the opportunity to work in his group.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publishing of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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