JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

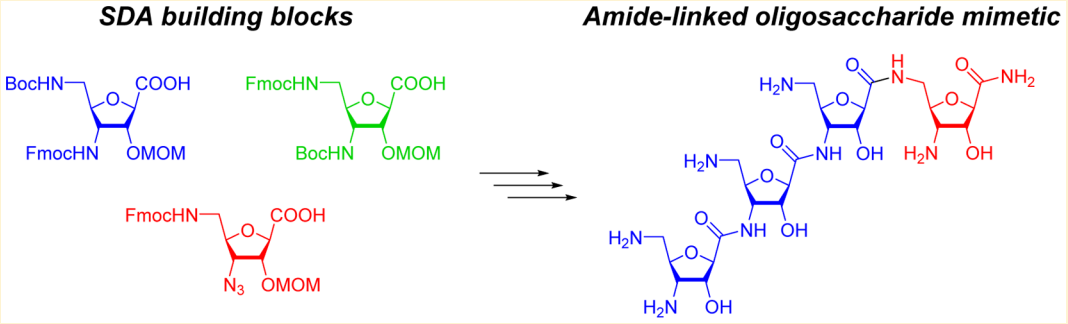
Orthogonally Protected Furanoid Sugar Diamino Acids for Solid-Phase Synthesis of Oligosaccharide Mimetics

Franklin John[†,‡] and Valentin Wittmann*[,†]

[†]Fachbereich Chemie and Konstanz Research School Chemical Biology (KoRS-CB), Universität Konstanz, 78457 Konstanz, Germany

[‡]Department of Chemistry, Sacred Heart College (M. G. University), Kochi 682013, India

Supporting Information



ABSTRACT: Sugar diamino acids (SDAs), which differ from the widely used sugar amino acids in the presence of a second amino group connected to the carbohydrate core, share structural features of both amino acids and carbohydrates. They can be used for the preparation of linear and branched amide-linked oligosaccharide mimetics. Such oligomers carry free amino groups, which are positively charged at neutral pH, in a spatially defined way and, thus, represent a potential class of aminoglycoside mimetics. We report here the first examples of orthogonally protected furanoid SDAs and their use in solid-phase synthesis. Starting from D-glucose, we developed a divergent synthetic route to three derivatives of 3,5-diamino-3,5-dideoxy-D-ribofuranose. These building blocks are compatible with solid-phase peptide synthesis following the 9-fluorenylmethoxycarbonyl (Fmoc) strategy, which we demonstrate by the synthesis of an SDA tetramer.

INTRODUCTION

Carbohydrates are involved in a myriad of biological processes, including cell−cell recognition, modulation of protein function, and many important biosynthetic pathways.[1] Structurally, carbohydrates represent highly diverse molecular scaffolds that offer several attachment points for introducing diversity, making them ideal templates for combinatorial synthesis and drug discovery.[2] The application of carbohydrates for the presentation of pharmacophores in a defined spatial arrangement was demonstrated in seminal studies by Hirschmann et al.[3] Sugar amino acids (SAAs) are special examples of carbohydrate-derived scaffolds that also have been used for the generation of pharmacophore-mapping combinatorial libraries.[4] SAAs are carbohydrate derivatives comprising at least one amino and one carboxy group connected to the carbohydrate core.[5] In nature, SAAs occur for example as cell-wall components in the form of neuraminic acid and muramic acid and as substructures of nucleoside antibiotics (e.g., ezomycin A,[6] gougerotin,[7] and aspiculamycin[8]) and of herbicides (e.g., hydantocidin[9]).

Sharing structural features of both amino acids and carbohydrates, SAAs can bridge the two classes of biopolymers formed from these building blocks. As such, SAAs have been applied for the preparation of peptidomimetics as well as amide-linked oligosaccharide mimetics. For example, SAAs can function as secondary-structure-inducing elements[10] and have been incorporated as turn mimetics in analogues of biologically active peptides, such as Leu-enkephalin,[10b,11] RGD-containing integrin antagonists,[12] somatostatin,[11,13] and gramicidin S.[14] Linear and cyclic SAA oligomers can adopt defined three-dimensional structures[15] and, thus, belong to the family of so-called foldamers.[16]

An attractive application of SAAs is the preparation of oligosaccharide mimetics in which the glycosidic linkages are replaced by amide bonds.[15d,17] If one takes advantage of the well-developed protocols for automated solid-phase peptide synthesis, such oligomers can be efficiently synthesized without the challenge of selectively forming new stereocenters as is required in oligosaccharide synthesis during every glycosylation step. Previously, we introduced a new class of pyranoid SAAs with an additional amino group, termed sugar diamino acids (SDAs).[18] We employed orthogonally protected SDA building blocks for the preparation of linear and branched oligosacchar-

Received: May 11, 2015
Published: July 10, 2015

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

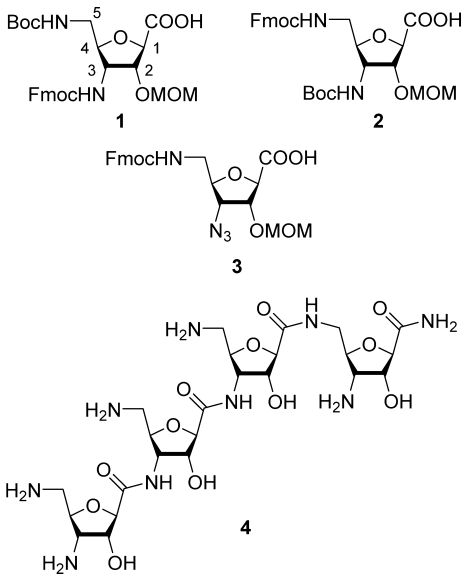
ide mimetics. Besides the opportunity to form branched structures, the additional amino groups can also be exploited for ligand−target interactions. In the case of the aminoglycoside antibiotics, the presentation of positive charges in a spatially defined way has been shown to be a key feature for achieving selectivity for certain RNA targets because of electrostatic complementarity.[19] Thus, oligomeric SDAs with their additional free amino groups represent a potential class of aminoglycoside mimetics. Such new antibiotics may give options for overcoming the worldwide emergence of antibiotic resistance.[20]

The major hurdle in the preparation of SDAs is the incorporation of one or both amino groups and their orthogonal protection to allow selective derivatization during oligomer synthesis. So far, only a few examples of SDAs, all featuring a pyranose ring, have been published.[18,21] We now report the first examples of furanoid SDAs and their use in solid-phase synthesis. Starting from D-glucose, we developed a divergent synthetic route to three derivatives of 3,5-diamino-3,5-dideoxy-D-ribofuranose. These building blocks are compatible with solid-phase peptide synthesis following the 9-fluorenylmethoxycarbonyl (Fmoc) strategy, which we demonstrate by the synthesis of an SDA tetramer.

RESULTS AND DISCUSSION

General Synthetic Considerations. Chart 1 depicts the three SDA building blocks **1**−**3** designed for use in solid-phase synthesis of oligosaccharide mimetics as well as tetramer **4** that we exemplarily synthesized. Using Fmoc groups as temporary amine protection, SDAs **1** and **2** allow the synthesis of oligomers connected via the amino group at positions 3 and 5,[22] respectively. Methoxymethyl (MOM) groups were chosen as permanent hydroxy protection. They can be easily removed under acidic conditions together with *tert*-butyloxycarbonyl (Boc) groups,[18] which were used as permanent protection of the second amino functionality. In addition, MOM groups show only a low level of interference with the reactivity of adjacent nucleophilic centers. They are less electron-withdrawing than ester protecting groups, such as acetyl and benzoyl residues, and they cannot undergo an irreversible O → N acyl shift. Furthermore, they are much smaller than benzyl ethers that have been shown to hinder acylation of adjacent amino groups in some cases.[15d] Building block **3** can be used for the synthesis of branched oligomers because the azide and the Fmoc group function as orthogonal protection[23] of the amines at positions 3 and 5. Whereas azides are stable in the presence of piperidine, which is commonly used for Fmoc cleavage, they are readily reduced to amines in the presence of Fmoc groups under neutral Staudinger reaction conditions.

Chart 1. Structures of SDA Building Blocks 1−3 and Oligosaccharide Mimetic 4



For the synthesis of SDA building blocks **1**−**3**, we developed a divergent route depicted in Scheme 1. The key intermediate for the synthesis of all three SDAs was C-furanoside **15** that was obtained from known[15c] isopropylidene-protected 3-azido-α-D-allofuranose **5**. Further functional group manipulation led to protected SDA **18** that served as a branching point of the synthesis to access building blocks **1** and **3**, the latter of which was subsequently transformed to building block **2**. In this way, the majority of synthetic steps was the same for all three compounds.

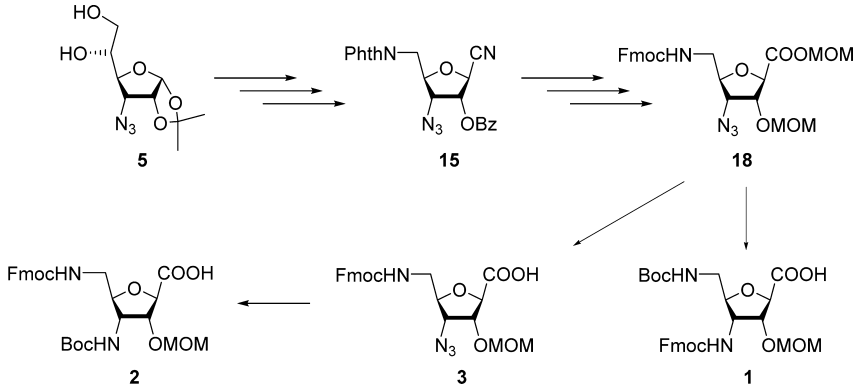
Synthesis of C-Glycosyl Cyanide 15. The synthesis of C-glycoside **15** started from literature-known[15c] 3-azido-α-D-allofuranose **5** in which the first amino function within the ribofuranose core structure was already present as an azido group. In a first attempt, we aimed to introduce the second amino group by reductive amination (Scheme 2). Diol **5** was oxidatively cleaved under mild conditions with sodium periodate, and the resultant aldehyde **6** was reacted with ammonium acetate and sodium cyanoborohydride.[24] The expected amine **7**, however, was not formed under these conditions; instead, the reaction resulted in the formation of secondary amine **8** likely via reaction of initially formed amine **7** with either aldehyde **6** or the iminium ion formed thereof. Attempts to trap intermediately formed amine **7** by Fmoc-Cl gave only low yields (up to 12% from **5**) of the Fmoc-protected derivative of **7**.

Because the reductive amination did not provide primary amine **7**, we turned our attention to the Gabriel synthesis[25] as an alternative method. Periodate cleavage of diol **5** followed by reduction with sodium borohydride afforded alcohol **9** in a yield of 77% over both steps (Scheme 3). The azido group was not affected under these conditions. Subsequently, **9** was converted to the corresponding tosylate **10**, which was treated with potassium phthalimide to give protected diamino ribose derivative **11**.

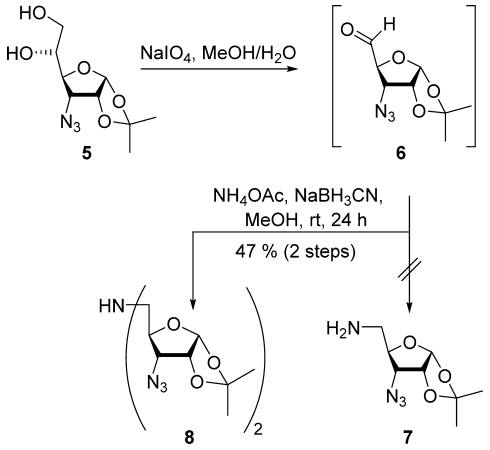
A critical step of the synthesis was the C-glycosylation reaction to introduce an anomeric cyano group as a precursor for the carboxylic acid. We decided to use an ester protecting group at position 2 of the diamino ribose to control the stereochemistry of this step by neighboring group participation. However, it had been reported that furanoses with an acetyl group at position 2 can lead to the formation of 1,2-*O*-cyanoethylidene derivatives.[26] Indeed, attempts to employ 1,2-di-*O*-acetyl-3-azido-3,5-dideoxy-5-phthalimido-D-ribofuranose, which was obtained in two steps from **11**, in a C-glycosylation reaction (TMS-CN, $SnCl_4$, CH_2Cl_2) failed and gave predominantly the corresponding 1,2-*O*-cyanoethylidene derivative (data not shown). Because the formation of cyanoethylidene derivatives can be suppressed by the use of benzoyl groups at position 2,[26c,f,27] we prepared 1-*O*-acetyl-2-*O*-benzoyl derivative **14** as a precursor for the C-glycosylation reaction. Acid-catalyzed methanolysis[27,28] of acetonide **11** resulted in methyl

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

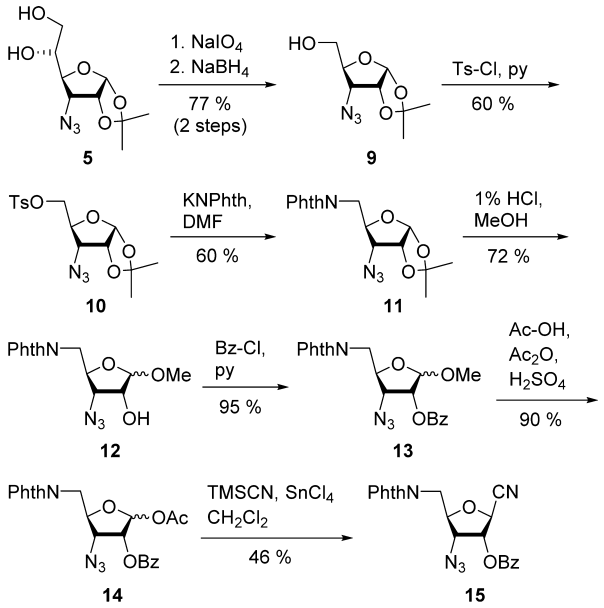
Scheme 1. General Synthetic Strategy for SDA Building Blocks 1−3



Scheme 2. Reductive Amination of Intermediate Aldehyde 6



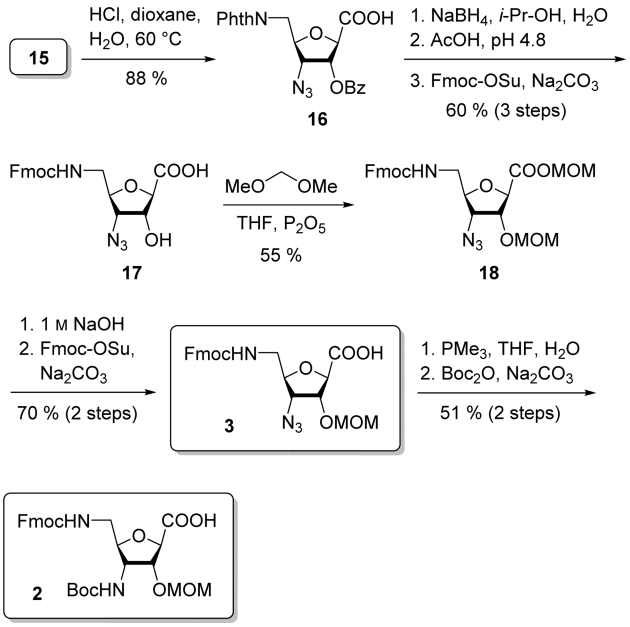
Scheme 3. Synthesis of Ribofuranosyl Cyanide 15



glycoside **12** in a yield of 72%. Benzoylation of **12** in pyridine afforded ribofuranoside **13** that was further converted into glycosyl acetate **14** by acetolysis in excellent yield over both steps. C-Glycosylation of **14** was achieved with TMS-CN employing $SnCl_4$ as a Lewis acid catalyst and dichloromethane as a solvent to give **15** in a yield of 46%. Formation of the 1,2-*O*-cyanobenzylidene derivative was not observed. Other catalysts, such as $BF_3{\cdot}OEt_2$ or $SnCl_2$, were also attempted for this conversion but resulted in lower yields.

Synthesis of SDA Building Blocks 1−3. For the synthesis of common intermediate **18**, glycosyl cyanide **15** was hydrolyzed at 60 °C under acidic conditions, resulting in the formation of carboxylic acid **16** in a yield of 88% without affecting any other functional groups (Scheme 4). Removal of

Scheme 4. Synthesis of SDA Building Blocks 2 and 3



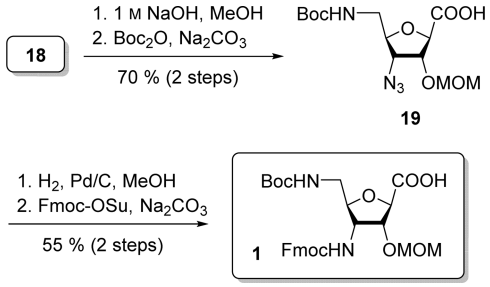
the phthaloyl group with preservation of the azide, however, was challenging. Hydrazine hydrate could not be used because of a known side reaction of the azide most probably caused by contaminating diimine.[29] Also, ethylenediamine, which had been developed as an alternative reagent for phthalimide cleavage that is compatible with azides,[29a,b,30] could not be employed because it resulted in decomposition of the starting material. We achieved the removal of the phthalimide group under mild conditions following a two-step one-flask procedure comprising $NaBH_4$ and subsequent acetic acid treatment.[31] The resulting primary amine was directly treated without any purification with Na_2CO_3, resulting in concomitant hydrolysis of the benzoic ester, and *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu) to furnish Fmoc-protected **17** in a

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

yield of 60% over three steps. The acid-labile MOM group was introduced by treatment with dimethoxymethane in the presence of P_2O_5 (avoiding the use of toxic MOM-Cl),[32] resulting in the formation of MOM ester derivative **18** in 55% yield. Subsequent ester hydrolysis of **18** under basic conditions and reprotection of the amine with Fmoc-OSu resulted in SDA **3** in a yield of 70%. A part of SDA **3** served as the starting material for the preparation of SDA building block **2**. For the reduction of the azide in the presence of the hydrogenolytically labile Fmoc group, we employed Staudinger conditions. Thus, treatment of **3** with trimethyl phosphine in THF and H_2O followed by protection of the free amine with di-*tert*-butyldicarbonate (Boc_2O) afforded **2** in an overall yield of 51%.

The synthesis of SDA building block **1**, finally, started from common intermediate **18** (Scheme 5). Saponification of **18**

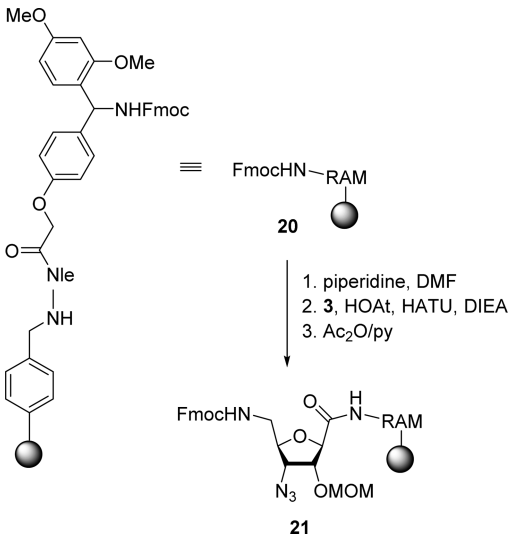
Scheme 5. Synthesis of SDA Building Block 1



resulted in ester hydrolysis and Fmoc removal to generate the free amine at position 5 that was subsequently Boc protected to yield intermediate compound **19**. Without further purification, crude **19** was subjected to hydrogenation with the Pd/C catalyst to reduce the azide to the free amine. Subsequent protection of the amine with Fmoc-OSu resulted in the formation of orthogonally protected SDA building block **1** in 39% yield starting from **18**.

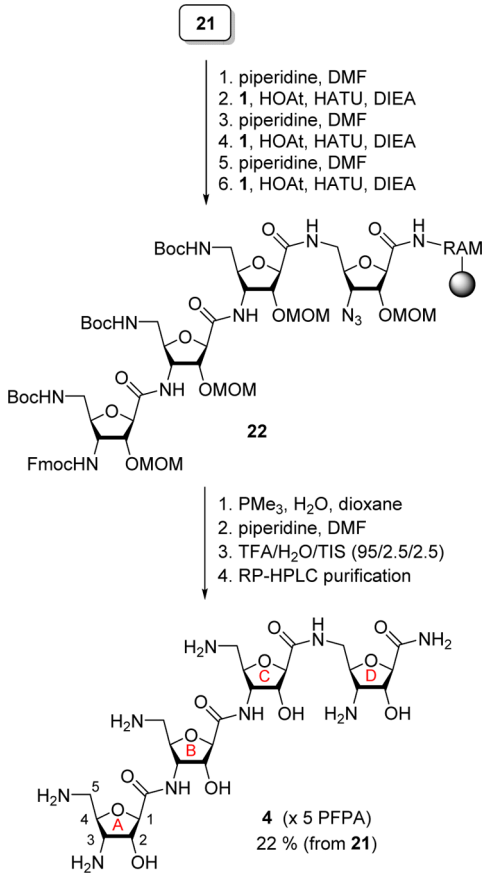
Solid-Phase Synthesis of Pseudo-oligosaccharide 4. To illustrate the application of the new SDA building blocks in solid-phase synthesis of amide-linked oligosaccharide mimetics following the standard Fmoc protocol,[33] we synthesized oligomer **4**. Rink amide AM (RAM) resin **20**[34] that is commonly used in peptide synthesis served as a solid support (Scheme 6). After removal of the Fmoc group with piperidine, SDA **3** was coupled as the first building block to give loaded resin **21**. Unreacted amino groups were capped by acetylation. SDA **3** is suited to the synthesis of branched oligomers. Although its use is not required for the synthesis of linear oligomer **4**, we employed **3** to demonstrate that the azido group is stable under the conditions of the solid-phase synthesis. Resin **21** then was stepwise elongated with building block **1** and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt) as coupling reagents[35] to give resin-bound tetramer **22** in six steps (Scheme 7). The azide group was reduced under Staudinger conditions using trimethyl phosphine in aqueous dioxane. After Fmoc deprotection with piperidine, the oligomer was cleaved from the resin with concomitant removal of the acid-labile Boc and MOM groups using a mixture of trifluoroacetic acid (TFA), triisopropyl silane (TIS), and water. Purification by RP-HPLC with addition of ion-pairing reagent pentafluoropropionic acid (PFPA)[36] to increase its otherwise low retention time gave **4** in a yield of

Scheme 6. Synthesis of Resin-Loaded SDA Building Block 21[a]



[a]DIEA represents diisopropyl ethylamine and Nle norleucine

Scheme 7. Solid-Phase Synthesis of Oligomer 4



22% starting from loaded resin **21** as pentakis(PFPA) salt at >90% purity. Oligomer **4** was thoroughly characterized by one- and two-dimensional ^1H and ^{13}C NMR spectroscopy to confirm its structure. Figure 1 shows the DQF-COSY spectrum, and ^1H and ^{13}C NMR chemical shifts are listed in Table 1.

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

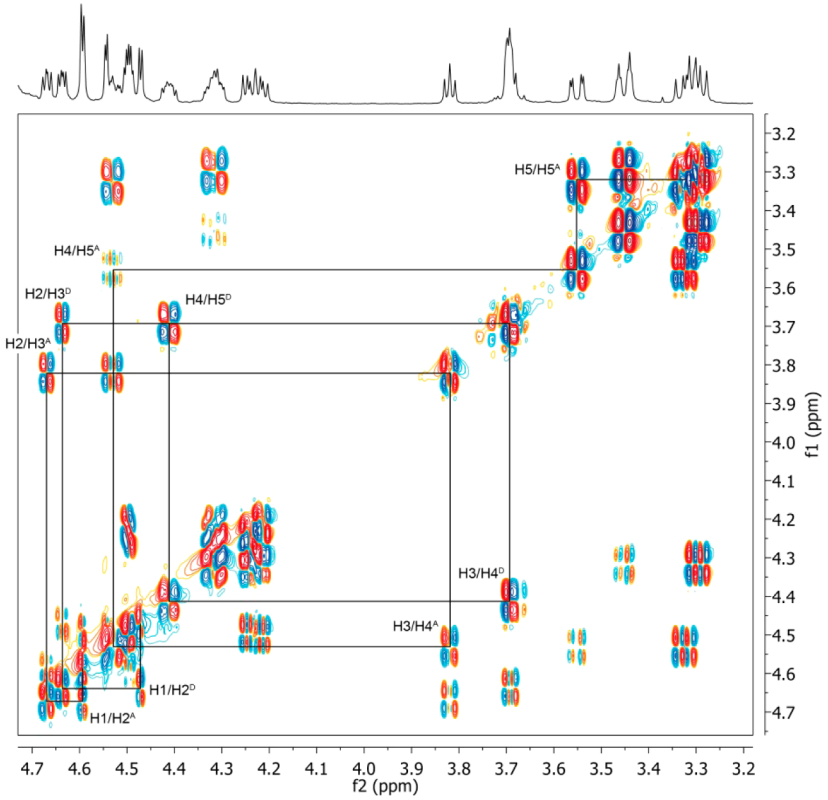


Figure 1. DQF-COSY spectrum (600 MHz, D_2O) of oligomer **4**. Proton assignments of rings A and D are exemplarily shown. For full assignments of peaks, see Table 1. For the definition of rings A–D, see Scheme 7.

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of Oligomer 4[a]

ring[b]	C(1)-H	C(2)-H	C(3)-H	C(4)-H	C(5)-H_2
A	4.59 (d, $J \approx 3.4$), 83.8 or 83.0	4.67 (dd, J = 6.3, 4.3), 71.9	3.82 ('t', J = 6.7), 53.0	4.53 (m, overl.), 76.6	3.55 (dd, J = 13.5, 2.9), 3.32 (m, overl.), 40.6
B[c]	4.59 (d, $J \approx 3.4$), 83.0 or 83.8	4.49 (m, overl.), 72.7	4.24 (dd, J = 5.7, 9.2), 52.9	4.34–4.29 (m, overl.), 76.9	3.47–3.43 (m, overl.), 3.32–3.28 (m, overl.), 40.9 or 40.7
C[c]	4.54 (d, $J \approx 2.7$), 84.1	4.51 (m, overl.), 72.7	4.22 (dd, J = 5.8, 9.3), 52.9	4.34–4.29 (m, overl.), 76.9	3.47–3.43 (m, overl.), 3.32–3.28 (m, overl.), 40.7 or 40.9
D	4.47 (d, J = 3.5), 82.8	4.64 (dd, J = 5.9, 3.6), 72.0	3.69 (m, overl.), 52.5	4.41 (ddd, J = 7.8, 6.0, 3.8), 78.9	3.71–3.66 (m, 2H, overl.), 40.2
A–D			δ_C of amides: 173.8, 172.1, 171.8, 171.2		

[a] ^{1}H and ^{13}C shifts determined in D_2O at 600 and 150 MHz, respectively. $^3J_{H,H}$ coupling constants given in hertz. Some signals could not be unambiguously assigned because of signal overlap (overl.). [b] For definition of rings A–D, see Scheme 7. [c] Rings B and C cannot be clearly distinguished and might be exchanged.

CONCLUSION

In summary, we have presented a divergent synthesis of orthogonally protected SDA building blocks **1**–**3**, which are the first examples of furanoid SDAs. The protecting group strategy adopted here allows the facile assembly of these derivatives on a solid support by a standard peptide coupling methodology. This has been demonstrated by the synthesis of amide-linked oligosaccharide **4**. The wide variety of possibilities in connecting these and previously published[18] SDAs to yield linear as well as branched structures makes them ideal building blocks for the combinatorial synthesis of oligosaccharide mimetics. Because oligomers made of SDAs represent a potential class of aminoglycoside mimetics, their synthesis opens new avenues for the development of new ligands to target RNA. Indeed, the synthesis of a library of SDA oligomers and their investigation as RNA ligands have been successfully conducted in our laboratory and will be published in due course.

EXPERIMENTAL SECTION

General Experimental Methods. Solvents for moisture sensitive reactions were distilled and dried according to standard procedures prior to use. All solvents for silica gel flash chromatography (FC) were distilled. 3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranose **5** was prepared according to a published procedure.[15c] Rink amide AM (RAM) resin **20** was obtained from Novabiochem (product no. 855120). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} aluminum sheets. For developing plates, ceric ammonium molybdate, $KMnO_4$, orcine, or ninhydrin was used. Flash column chromatography (FC) was performed on Macherey-Nagel silica gel 60 (0.04–0.063 mm, 230–400 mesh). NMR spectra were recorded at 298 K. Chemical shifts are referenced to the solvent signal ($CDCl_3$, δ_H = 7.26 and δ_C = 77.0; DMSO-d_6, δ_H = 2.5 and δ_C = 39.5). Optical rotations were determined at 25 °C with a 1 dm cell. Melting points are uncorrected.

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477–7485

*3-Azido-3-deoxy-1,2-O-isopropylidene-α-D-ribofuranose (**9**).* To a stirred solution of 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranose **5** (493 mg, 2.01 mmol) in MeOH (7 mL) and H_2O (10 mL) was gradually added $NaIO_4$ (516 mg, 2.4 mmol) at 0 °C, and the mixture was stirred for 4 h. The reaction mixture was diluted with MeOH (8 mL); the inorganic salts were filtered off, and the filtrate was evaporated to obtain a light yellow syrup. The crude product was dissolved in MeOH (5 mL) and THF (2 mL). $NaBH_4$ (86 mg, 2.22 mmol) was added and the reaction mixture stirred for 3 h at 0 °C and filtered. The solvent was evaporated and the residue purified by FC (petroleum ether/ethyl acetate, 1:2) to give **9** (334 mg, 1.55 mmol, 77%) as a white solid: mp 54−56 °C; R_f = 0.39 (petroleum ether/ethyl acetate, 1:2); $[\alpha]_D^{25}$ +58.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.77 (d, 1H, *J* = 3.7 Hz), 4.72 (t, 1H, *J* = 4.2 Hz), 4.10 (m, 1H), 4.00 (dd, 1H, *J* = 12.3, 2.2 Hz), 3.70 (dd, 1H, *J* = 12.5, 2.7 Hz), 3.52 (dd, 1H, *J* = 9.4, 4.5 Hz) 1.56 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 113.3, 104.2, 80.2, 78.2, 60.3, 59.5, 26.4; ESI-MS $[M + H]^+$ calcd for $C_8H_{14}N_3O_4$ *m*/*z* 216.1, found *m*/*z* 215.0. Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found: C, 44.74; H, 6.13; N, 19.34.

*3-Azido-3-deoxy-1,2-O-isopropylidene-5-O-tosyl-α-D-ribofuranose (**10**).* To a solution of **9** (333 mg, 1.55 mmol) in dry pyridine (4 mL) was added tosyl chloride (413 mg, 2.17 mmol) at 0 °C, and the mixture was stirred overnight. The reaction was quenched with water and the solvent evaporated. The crude product was purified by FC (petroleum ether/ethyl acetate, 2:1) to give **10** (342 mg, 0.93 mmol, 60%) as white crystals: mp 71−73 °C; R_f = 0.44 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D^{25}$ +42.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.78 (m, 2H), 7.35 (m, 2H), 5.72 (d, 1H, *J* = 3.6 Hz), 4.69 (t, 1H, *J* = 4.3 Hz), 4.31−4.10 (m, 3H), 3.49 (dd, 1H, *J* = 9.3, 4.5 Hz), 2.42 (s, 3H), 1.50 (3, 3H), 1.41 (3, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 145.2, 132.6, 130.3, 127.9, 113.4, 104.2, 79.8, 75.7, 67.0, 60.3, 26.4, 21.6; MALDI-MS $[M + Na]^+$ calcd for $C_{15}H_{19}N_3NaO_6S$ *m*/*z* 392.1, found *m*/*z* 392.8. Anal. Calcd for $C_{15}H_{19}N_3O_6S$: C, 48.77; H, 5.18; N, 11.38. Found: C, 48.61; H, 5.19; N, 11.34.

*3-Azido-3,5-dideoxy-1,2-O-isopropylidene-5-phthalimido-α-D-ribofuranose (**11**).* A solution of **10** (2.5 g, 6.77 mmol) and potassium phthalimide (1.5 g, 8.12 mmol) in dry DMF (20 mL) was stirred at 70 °C for 24 h. After addition of H_2O (30 mL), the reaction mixture was extracted with CH_2Cl_2 (2 × 50 mL). The organic layer was successively washed with 5% aqueous NaOH (20 mL) and brine and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by FC (petroleum ether/ethyl acetate, 1:1) to yield **11** (1.4 g, 4.07 mmol, 60%) as a white solid: mp 148−151 °C; R_f = 0.5 (petroleum ether/ethyl acetate, 1:1); $[\alpha]_D^{25}$ +47.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.87 (m, 2H), 7.74 (m, 2H), 5.80 (d, 1H, *J* = 3.7 Hz), 4.72 (t, 1H, *J* = 4.3 Hz), 4.30 (m, 1H), 3.98 (m, 2H), 3.32 (dd, 1H, *J* = 9.7, 4.7 Hz), 1.52 (3, 3H), 1.31 (3, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.1, 134.1, 132.0, 123.5, 113.3, 104.2, 80.1, 74.9, 63.4, 39.0, 26.5; MALDI-MS $[M + Na]^+$ calcd for $C_{16}H_{16}N_4O_5Na$ *m*/*z* 367.1, found *m*/*z* 366.7. Anal. Calcd for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27. Found: C, 55.95; H, 4.94; N, 16.55.

*Methyl 3-Azido-3,5-dideoxy-5-phthalimido-D-ribofuranoside (**12**).* To a stirred solution of **11** (1.7 g, 4.94 mmol) in MeOH (15 mL) was added acetyl chloride (0.2 mL) at room temperature and the mixture stirred for 5 h. The solution was neutralized with pyridine (3 mL) and the solvent evaporated. The crude product was purified by FC (petroleum ether/ethyl acetate, 2:1) to give **12** (1.13 g, 3.55 mmol, 72%, ≈1:12 α:β) as a white foam: R_f = 0.21 (petroleum ether/ethyl acetate, 2:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (m, 2H), 7.72 (m, 2H), 4.89 (d, 1H, *J* = 4.6 Hz, α isomer), 4.79 (s, 1H, β isomer), 4.30 (m, 1H, β isomer), 4.14 (d, 1H, *J* = 4.3 Hz, β isomer), 4.06 (dd, 1H, *J* = 7.9, 4.4 Hz, β isomer), 3.98 (m, 2H, β isomer), 3.44 (s, 3H, α isomer), 3.29 (s, 3H, β isomer); ^{13}C NMR (150 MHz, $CDCl_3$) δ (β isomer) 168.2, 134.2, 131.9, 123.6, 108.0, 78.3, 75.6, 64.4, 55.3, 40.7; MALDI-MS $[M + Na]^+$ calcd for $C_{14}H_{14}N_4O_5Na$ *m*/*z* 341.1, found *m*/*z* 341.7. Anal. Calcd for $C_{14}H_{14}N_4O_5$: C, 52.83; H, 4.43; N; 17.60. Found: C, 53.05; H, 4.76; N, 7.68.

*Methyl 3-Azido-2-O-benzoyl-3,5-dideoxy-5-phthalimido-D-ribofuranoside (**13**).* To a solution of **12** (8.3 g, 26.1 mmol) in pyridine (60 mL) was added dropwise benzoyl chloride (15 mL, 130 mmol) at room temperature and the reaction mixture stirred for 3 h. The solvent was evaporated, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine (twice), dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by FC (petroleum ether/ethyl acetate, 2:1) to give **13** (10.5 g, 24.85 mmol, 95%, ≈1:4 α:β) as a colorless gum: R_f = 0.4 (petroleum ether/ethyl acetate, 2:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (m, 1H), 8.04 (m, 1H), 7.89 (m, 2H), 7.75 (m, 2H), 7.59 (m, 1H), 7.46 (m, 2H), 5.44 (d, 1H, *J* = 4.6 Hz, β isomer), 5.24 (d, 1H, *J* = 4.3 Hz, α isomer), 5.19 (dd, 1H, *J* = 7.9, 4.4 Hz, α isomer), 4.95 (s, 1H, β isomer), 4.41 (m, 1H, β isomer), 4.30 (m, 1H, α isomer), 4.24 (dd, 1H, *J* = 8.3, 4.6 Hz, β isomer), 4.17 (m, 1H, α isomer), 4.04 (m, 2H, β isomer), 3.41 (s, 3H, α isomer), 3.36 (s, 3H, β isomer); ^{13}C NMR (150 MHz, $CDCl_3$) δ (β isomer) 168.3, 165.4, 134.3, 133.7, 130.1, 129.0, 128.6, 128.5, 123.5, 106.1, 78.4, 76.6, 62.7, 55.4, 40.7; MALDI-MS $[M + Na]^+$ calcd for $C_{21}H_{18}N_4O_6Na$ *m*/*z* 445.1, found *m*/*z* 445.6. Anal. Calcd for $C_{21}H_{18}N_4O_6$: C, 59.71; H, 4.30; N, 13.26. Found: C, 59.83; H, 4.48; N, 12.38.

*1-O-Acetyl-3-azido-2-O-benzoyl-3,5-dideoxy-5-phthalimido-D-ribofuranose (**14**).* Compound **13** (18.2 g, 43.09 mmol) was dissolved in acetic acid (172 mL). Acetic anhydride (40.5 mL, 431.2 mmol) and concentrated H_2SO_4 (11.5 mL, 215 mmol) were added to the solution, which was then stirred for 1 h. The reaction mixture was poured into a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by FC (petroleum ether/ethyl acetate, 2:1) to give **14** (17.47 g, 38.8 mmol, 90%, ≈16:1 α:β) as a white solid: R_f = 0.3 (petroleum ether/ethyl acetate, 2:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.87 (m, 2H), 7.73 (m, 2H), 7.56 (m, 1H), 7.45 (m, 2H), 6.55 (d, 1H, *J* = 4.5 Hz, α isomer), 6.24 (s, 1H, β isomer), 5.55 (d, 1H, *J* = 4.5 Hz, β isomer), 5.49 (dd, 1H, *J* = 7.5, 4.3 Hz, α isomer), 4.49 (m, 1H, α isomer), 4.19 (m, 1H, α isomer), 4.08−3.91 (m, 2H, α isomer), 2.07 (s, 3H, α isomer), 1.91 (s, 3H, β isomer); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (α isomer) 169.1, 168.1, 165.5, 134.3, 133.5, 131.7, 129.9, 128.9, 128.7, 123.8, 94.1, 80.4, 72.3, 60.4, 39.6, 20.7; MALDI-MS $[M + Na]^+$ calcd for $C_{22}H_{18}NaN_4O_7$ *m*/*z* 473.1, found *m*/*z* 472.7. Anal. Calcd for $C_{22}H_{18}N_4O_7$: C, 58.67; H, 4.03; N, 12.44. Found: C, 58.85; H, 4.12; N, 12.41.

*3-Azido-2-O-benzoyl-1-cyano-5-phthalimido-1,3,5-trideoxy-β-D-ribofuranose (**15**).* To a solution of **14** (15 g, 33.3 mmol) and TMS-CN (25 mL, 200 mmol) in anhydrous CH_2Cl_2 (150 mL) was added dropwise a 1 M solution of $SnCl_4$ in CH_2Cl_2 (40 mL) at room temperature. The reaction mixture was refluxed for 5 h and poured into a saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (4 × 200 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and evaporated to obtain a crude residue. It was purified by FC (petroleum ether/ethyl acetate, 2:1) to yield **15** (6.39 g, 15.32 mmol, 46%) as a pale yellow solid: mp 168−170 °C; R_f = 0.4 (petroleum ether/ethyl acetate, 2:1); $[\alpha]_D^{25}$ +44.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (m, 2H), 7.91 (m, 2H), 7.76 (m, 2H), 7.63 (m, 1H), 7.48 (m, 2H), 5.77 (m, 1H), 4.78 (d, 1H, *J* = 2.2 Hz), 4.37 (m, 1H), 4.29 (m, 1H), 4.16−4.08 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 164.7, 134.4, 134.1, 131.7, 130.0, 128.7, 128.0, 123.6, 115.4, 79.8, 75.8, 69.8, 63.0, 38.5; MALDI-MS $[M + Na]^+$ calcd for $C_{21}H_{15}NaN_5O_5$ *m*/*z* 440.1, found *m*/*z* 440.1. Anal. Calcd for $C_{21}H_{15}N_5O_5$: C, 60.43; H, 3.62; N, 16.78. Found: C, 60.43; H, 3.62; N, 16.69.

*3-Azido-2-O-benzoyl-5-phthalimido-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid (**16**).* To a solution of **15** (10.60 g, 25.4 mmol) in a dioxane/water solvent (10:1, 110 mL) was added HCl (4 M in dioxane, 100 mL), and the mixture was heated to 60 °C for 24 h and the solvent evaporated. The residue was dissolved in 1 N HCl and extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and dried. The crude residue was purified by FC (CH_2Cl_2/MeOH, 9:1) to yield **16** (9.79 g, 22.4 mmol, 88%) as a pale yellow foam: R_f = 0.3 (CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{25}$

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

+44.8 (*c* 0.5, $CHCl_3$); ^1H NMR (250 MHz, DMSO-d_6) δ 7.99−7.96 (m, 2H), 7.89−7.82 (m, 4H), 7.71−7.64 (m, 1H), 7.56−7.50 (m, 2H), 5.70 (m, 1H), 4.36 (d, J = 1.8 Hz, 1H), 4.28 (dd, J = 8.3, 4.8 Hz, 1H), 4.11−4.03 (m, 3H); ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 171.3, 167.8, 164.7, 134.3, 133.6, 131.5, 129.2, 128.8, 128.7, 122.9, 82.3, 77.3, 76.6, 63.4, 39.8; MALDI-MS $[M + H]^+$ calcd for $C_{21}H_{17}N_4O_7$ m/z 437.1, found m/z 437.1. Anal. Calcd for $C_{21}H_{16}N_4O_7$: C, 57.80; H, 3.70; N, 12.84. Found: C, 57.98; H, 3.68; N, 12.81.

*3-Azido-5-(9-fluorenylmethoxycarbonylamino)-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid (**17**).* To a stirred solution of **16** (2.1 g, 6.7 mmol) in 2-propanol (62 mL) and H_2O (10 mL) was added $NaBH_4$ (1.27 g, 33.5 mmol), and the solution was then stirred for 24 h. TLC indicated complete consumption of the starting material. Glacial acetic acid (7.2 mL) was added carefully to adjust the pH to 4.8, and when the foaming subsided, the reaction mixture was stirred at 80 °C for 15 h. The solvent was evaporated, and the crude residue was used in the next step without any purification. The crude amine was dissolved in an acetone/H_2O solvent (1:1, 6 mL). A 1 M Na_2CO_3 solution (2.8 mL) was added, and the mixture was stirred for 5 h to hydrolyze the benzoyl ester. To this solution was added Fmoc-OSu (950 mg, 2.83 mmol) dissolved in acetone (5 mL), and the solution was stirred for 8 h. Acetone was evaporated, and the reaction mixture was acidified to pH 3 with 1 N HCl. The reaction mixture was extracted with ethyl acetate (5 × 30 mL). The combined organic phases were washed twice with H_2O and dried over Na_2SO_4, and the solvent was evaporated. The crude residue was purified by FC (CH_2Cl_2/MeOH, 9:1) to yield **17** (1.6 g, 3.8 mmol, 60%) as a white solid: mp 140−142 °C; R_f = 0.3 (CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{25}$ +41.7 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6) δ 8.35 (m, 2H), 8.20 (m, 2H), 7.86 (m, 2H), 7.78 (m, 2H), 7.20 (br s, 1H), 5.18 (m, 1H), 4.81−4.6 (m, 4H), 4.25 (m, 2H), 3.78 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.5, 168.1, 153.5, 152.0, 138.5, 138.2, 137.1, 132.5, 94.8, 91.3, 88.2, 78.0, 73.1, 58.1, 52.3; ESI-MS $[M + H]^+$ $C_{21}H_{21}N_4O_6$ calcd for m/z 425.2, found m/z 425.4. Anal. Calcd for $C_{21}H_{20}N_4O_6$: C, 59.43; H, 4.75; N, 13.20. Found: C, 59.41; H, 4.73; N, 12.82.

*3-Azido-5-(9-fluorenylmethoxycarbonylamino)-2-O-methoxymethyl-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid Methoxymethyl Ester (**18**).* To a stirred solution of **17** (2.4 g, 5.66 mmol) in formaldehyde dimethyl acetal (120 mL) and THF (5 mL) was added P_2O_5 (1.2 g) in portions, and the mixture was stirred for 3 h and then transferred to a flask. The remaining solids were taken up in Na_2CO_3 (1 M, 30 mL), and the aqueous layer was then extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with a saturated $NaHCO_3$ solution and H_2O. After it had been dried over Na_2SO_4 and the liquid evaporated, the crude product was purified by FC (petroleum ether/ethyl acetate, 1:1) to give **18** (1.595 g, 3.1 mmol, 55%) as a colorless gum: R_f = 0.35 (petroleum ether/ethyl acetate, 1:1); $[\alpha]_D^{25}$ −0.7 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, 2H, J = 6.0 Hz), 7.65−7.60 (m, 2H), 7.41−7.39 (m, 2H), 7.33−7.31 (m, 2H), 5.85 (br m, 1H), 5.36−5.26 (m, 2H), 4.83−4.78 (m, 2H), 4.58 (d, J = 1.8 Hz, 1H), 4.46- 4.41 (m, 3H), 4.27−4.23 (m, 2H), 3.65−3.43 (m, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.8, 143.8, 141.3, 127.7, 127.1, 125.2, 124.8, 119.9, 96.2, 91.6, 81.1, 80.2, 79.8, 66.8, 60.4, 58.1, 56.1, 47.3, 41.4; HRMS (ESI-TOF) $[M + H]^+$ calcd for $C_{25}H_{29}N_4O_8^+$ m/z 513.1980, found m/z 513.1994.

*3-Azido-5-(9-fluorenylmethoxycarbonylamino)-2-O-methoxymethyl-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid (**3**).* A solution of **18** (2 g, 3.9 mmol) in MeOH (10 mL) was stirred with 1 N NaOH (11.7 mL) for 2 h. The reaction mixture was neutralized with 1 N HCl (12 mL), and the solvent was evaporated in vacuum to give the crude amino acid. The crude product was dissolved in an acetone/H_2O solvent (1:2, 8 mL), and a 1 M Na_2CO_3 solution (3 mL) was added. To this solution was added Fmoc-OSu (950 mg, 2.83 mmol) in acetone (5 mL), and the mixture was stirred for 8 h. Acetone was evaporated, and the reaction mixture was acidified to pH 3 with 1 N HCl. The reaction mixture was extracted with ethyl acetate (5 × 30 mL). The combined organic phases were washed with H_2O (twice) and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The crude residue was purified by FC (CH_2Cl_2/MeOH, 9:1) to yield **3** (1.26 g, 2.7 mmol, 70%) as a white solid: R_f = 0.25 (CH_2Cl_2/MeOH, 9:1); mp 78−80 °C; $[\alpha]_D^{25}$ +15.4 (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6) δ 8.3 (br s, 1H), 7.86 (m, 2H), 7.69 (m, 2H), 7.39 (m, 2H), 7.31 (m, 2H), 4.83 (d, J = 6.6 Hz, 1H), 4.70 (d, J = 6 Hz, 1H), 4.39 (m, 1H), 4.23−4.21 (m, 4H), 4.07−4.00 (m, 2H), 3.76 (m, 1H), 3.34 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 173.3, 156.5, 143.8, 140.6, 127.5, 127.0, 125.2, 120.0, 94.8, 82.6, 79.9, 78.4, 65.7, 61.0, 55.1, 46.6, 42.3; MALDI-MS $[M + Na]^+$ calcd for $C_{23}H_{24}N_4O_7Na$ m/z 491.1, found m/z 491.0. Anal. Calcd for $C_{23}H_{24}N_4O_7$: C, 58.97; H, 5.16; N, 11.96. Found: C, 58.91; H, 5.14; N, 11.91.

*3-(tert-Butoxycarbonylamino)-5-(9-fluorenylmethoxycarbonylamino)-2-O-methoxymethyl-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid (**2**).* To a stirred solution of **3** (700 mg, 1.49 mmol) in THF (10 mL) were added PMe_3 (1 M in THF, 7.5 mL) and H_2O (15 mL), and then the mixture was stirred for 5 h. The solvent was evaporated, and toluene was evaporated several times from the remainder. The crude amine [500 mg, brownish liquid, R_f = 0.35 (MeCN/H_2O, 4:1)] was dissolved in H_2O (10 mL). A 1 M Na_2CO_3 solution (3 mL) and then a solution of Boc_2O (480 mg, 2.2 mmol) in dioxane (3 mL) were added. After having been stirred for 5 h, the solution was concentrated and acidified to pH 3 by addition of 1 N HCl. The product was extracted with $CHCl_3$ (4 × 20 mL); the combined organic phases were dried (Na_2SO_4) and concentrated, and the crude residue was purified by FC (ethyl acetate/MeOH, 9:1) to give **2** (412 mg, 0.76 mmol, 51%) as a white solid: R_f = 0.17 (ethyl acetate/MeOH, 9:1); mp 92−94 °C; $[\alpha]_D^{25}$ +15.4 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6) δ 7.89 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.0 Hz, 2H), 7.45−7.30 (m, 4H), 7.10 (m, 1H), 4.72 (m, 1H), 4.59 (m, 1H), 4.43 (m, 1H), 4.21 (m, 4H), 4.0 (m, 2H), 3.81 (m, 1H), 3.27 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.7, 158.4, 144.1, 140.9, 127.9, 127.4, 125.5, 120.3, 95.3, 80.9, 79.4, 78.5, 77.9, 66.1, 65.7, 55.3, 53.8, 46.9, 28.4; ESI-MS $[M - H]^-$ calcd for $C_{28}H_{33}N_2O_9$ m/z 541.2, found m/z 541.1. Anal. Calcd for $C_{28}H_{34}N_2O_9$: C, 61.98; H, 6.32; N, 5.16. Found: C, 61.72; H, 6.31; N, 5.14.

*5-(tert-Butoxycarbonylamino)-3-(9-fluorenylmethoxycarbonylamino)-2-O-methoxymethyl-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic Acid (**1**).* A solution of **18** (2.3 g, 4.49 mmol) in MeOH (10 mL) was treated with 1 N NaOH (12 mL) and stirred for 2 h at room temperature. The reaction mixture was neutralized with 1 N HCl, and the solvent was evaporated. The crude amino acid was dissolved in a dioxane/H_2O solvent (1:4, 10 mL). A 1 M Na_2CO_3 solution (3 mL) and a solution of Boc_2O (1.18 g, 5.39 mmol) in dioxane (5 mL) were added, and the mixture was stirred for 5 h at room temperature. The reaction mixture was acidified with 1 N HCl to pH 3 and extracted with $CHCl_3$ (4 × 20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to obtain 3-azido-5-(*tert*-butoxycarbonylamino)-2-*O*-methoxymethyl-1,3,5-trideoxy-β-D-ribofuranose-1-carboxylic acid (**19**) as a white solid [1.09 g, 3.14 mmol, 70%, R_f = 0.17 (CH_2Cl_2/MeOH, 4:1)] that was used in the next step without any purification. Crude **19** (1 g, 2.89 mmol) was dissolved in anhydrous MeOH (15 mL). After addition of dry 10% palladium on a carbon catalyst (two spatula tips), the reaction mixture was vigorously stirred under H_2 (1 atm) at room temperature for 40 min. The mixture was filtered through Celite and the solvent evaporated. The crude oily liquid [0.9 g, 2.81 mmol, R_f = 0.21 (CH_2Cl_2/MeOH, 4:1)] was dissolved in an acetone/H_2O solvent (1:1, 10 mL). A solution of Fmoc-OSu (1.14 g, 3.37 mmol) in acetone (8 mL) was added, and the mixture was stirred overnight. The reaction mixture was acidified with 1 N HCl to pH 3, and the product was extracted with $CHCl_3$ (4 × 20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. The obtained crude product was purified by FC (CH_2Cl_2/MeOH, 9:1) to yield **1** (860 mg, 1.59 mmol, 55%) as a white solid: R_f = 0.20 (CH_2Cl_2/MeOH, 9:1); mp 75−78 °C; $[\alpha]_D^{25}$ +15.8 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6) δ 7.87 (m, 2H), 7.69 (m, 2H), 7.40 (m, 2H), 7.33 (m, 2H), 4.69 (d, J = 6.6 Hz, 1H), 4.51 (d, J = 6.6 Hz, 1H), 4.27 (m, 2H), 4.19−4.11 (m, 4H), 4.01 (m, 1H), 3.20−3.14 (m, 4H), 1.35 (s, 9H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.9, 156.1, 144.0, 140.8, 127.7, 127.2, 125.3, 120.2, 94.5, 78.8, 77.7, 77.4, 77.2, 65.7, 59.9, 54.9, 46.8, 28.4; ESI-MS $[M - H]^-$ calcd for

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

$C_{28}H_{33}N_2O_9$ m/z 541.2, found m/z 541.7. Anal. Calcd for $C_{28}H_{34}N_2O_9$: C, 61.98; H, 6.32; N, 5.16. Found: C, 61.71; H, 6.29; N, 5.14.

Solid-Phase Synthesis of Oligomer **4**. Solid-phase synthesis was conducted in a disposable fritted syringe according to standard Fmoc protocols[33] starting from Rink amide-AM-resin **20** (152 mg, loading density of 0.96 mmol g^{-1}). For Fmoc deprotection, the resin was swollen for 5 min in DMF. The solvent was removed and the resin shaken with 20% piperidine in DMF (1 mL per 100 mg of resin) (1 × 3 min, 1 × 10 min). For loading of resin, after Fmoc deprotection the resin was washed with DMF (10 × 1 min). SDA **3** (137 mg, 0.29 mmol), HOAt (40 mg, 0.29 mmol), HATU (106 mg, 0.28 mmol), and DIEA (100 μL, 0.59 mmol) were mixed in *N*-methylpyrrolidin-2-one (NMP) and CH_2Cl_2 [3:1 (v/v), 1 mL], taken into the syringe, and shaken for 3 h. Subsequently, the resin was washed with DMF (10 × 1 min). For capping, the resin was shaken with an Ac_2O/pyridine mixture (1:3, 0.5 mL per 100 mg of resin) for 15 min and then washed with DMF (10 × 1 min). For determination of loading density, the resin was washed with CH_2Cl_2 (2 × 1 min) and dried under vacuum to afford resin-bound SDA building block **21** (168 mg). A small amount of dry resin (4 mg) was taken out and treated with 20% piperidine in DMF (10 mL) for 15 min. The UV absorbance at 301 nm of the cleavage product (piperidine−dibenzofulvene adduct) was determined using a 1 cm cuvette, and its concentration was calculated using an extinction coefficient (ε_{301}) of 8100 L mol^{-1} cm^{-1}. The loading density of resin **21** was calculated to be 0.39 mmol g^{-1}. For further coupling steps with SDA **1**, first, Fmoc deprotection was conducted with a piperidine/DMF mixture as described above. Then, SDA building block **1** (2 equiv relative to resin loading), HOAt (2 equiv), HATU (1.9 equiv), and DIEA (4 equiv) were mixed in NMP (0.5−1 mL), taken into the syringe, and shaken for 2−3 h. The resin was washed with DMF (10 × 1 min), and capping was conducted as described above. A Kaiser test[37] was performed with a small resin sample to ensure complete coupling. After three coupling steps with SDA **1**, the resin was washed with DMF (10 × 1 min) and CH_2Cl_2 (2 × 3 min) and dried under vacuum to give resin-bound oligomer **22**. For Staudinger reduction on the solid phase, to reduce the azido group present in **22**, the resin was suspended in a mixture of dioxane (0.4 mL) and water (0.1 mL). PMe_3 (1 M in THF, 6 equiv) was added, and the capped syringe was shaken for 2 h. The resin was washed with anhydrous dioxane (five times) to remove the excess PMe_3. Subsequently, Fmoc deprotection was conducted with a piperidine/DMF mixture as described above, and the resin was thoroughly washed with DMF and CH_2Cl_2. For peptide cleavage, the resin was shaken with a freshly prepared TFA/TIS/water mixture [95:2.5:2.5 (v/v/v)] for 6 h and filtered off. The cleavage solution was added dropwise to a 20-fold excess of ice cold *tert*-butyl-methyl ether and kept in the freezer overnight to precipitate the SDA oligomer. In parallel, the resin was washed with 2−3 mL of water and lyophilized. The precipitated crude product was removed from the cleavage solution by centrifugation (10000 rpm, 20 min). The obtained pellet was resuspended in cold *tert*-butyl-methyl ether and centrifuged again. The pellet and the lyophilized product were combined and lyophilized again from water. For purification, the crude product was dissolved in a solution of 0.13% PFPA in water and purified by RP-HPLC (Nucleosil C18 column from Knauer, analytical scale of 250 mm × 4 mm, flow rate of 1 mL min^{-1}, preparative scale of 250 mm × 8 mm, flow rate of 6 mL min^{-1}) using a gradient from 1 to 50% B in A over 30 min (A being 0.13% PFPA in water and B being 0.13% PFPA in MeCN). After RP-HPLC purification, SDA oligomer **4** was lyophilized to obtain the corresponding pentakis(PFPA) salt (9 mg, 22% from **21**). Analytical RP-HPLC: t_R = 12.5 min. ^1H and ^{13}C NMR data determined in D_2O at 600 MHz (^1H) and 150 MHz (^{13}C), respectively, are listed in Table 1: ESI-MS $[M + H]^+$ calcd for $C_{24}H_{44}N_9O_{12}$ m/z 650.3, found m/z 650.3.

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra of newly synthesized compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01049.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mail@valentin-wittmann.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 579), the German Academic Exchange Service (DAAD), and the University of Konstanz. We thank Claudia Meßmer for assisting in the large scale synthesis of compound **5** and Anke Friemel for the measurement of NMR spectra.

■ DEDICATION

Dedicated to Professor Richard R. Schmidt on the occasion of his 80th birthday.

■ REFERENCES

(1) (a) *Essentials of Glycobiology*, 2nd ed.; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor Laboratory Press: Plainview, NY, 2009. (b) *Carbohydrate Recognition: Biological Problems, Methods, and Applications*; Wang, B., Boons, G.-J., Eds.; John Wiley & Sons: Hoboken, NJ, 2011.

(2) (a) Sofia, M. J. *Mol. Diversity* **1998**, *3*, 75−94. (b) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2503−2505. (c) Wong, C.-H.; Ye, X.-S.; Zhang, Z. *J. Am. Chem. Soc.* **1998**, *120*, 7137−7138.

(3) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B., III; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. *J. Am. Chem. Soc.* **1992**, *114*, 9217−9218.

(4) (a) Sofia, M. J.; Hunter, R.; Chan, T. Y.; Vaughan, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802−2803. (b) Sofia, M. J.; Allanson, N.; Hatzenbuhler, N. T.; Jain, R.; Kakarla, R.; Kogan, N.; Liang, R.; Liu, D.; Silva, D. J.; Wang, H.; Gange, D.; Anderson, J.; Chen, A.; Chi, F.; Dulina, R.; Huang, B.; Kamau, M.; Wang, C.; Baizman, E.; Branstrom, A.; Bristol, N.; Goldman, R.; Han, K.; Longley, C.; Midha, S.; Axelrod, H. R. *J. Med. Chem.* **1999**, *42*, 3193−3198. (c) Ghosh, M.; Dulina, R. G.; Kakarla, R.; Sofia, M. J. *J. Org. Chem.* **2000**, *65*, 8387−8390. (d) Edwards, A. A.; Ichihara, O.; Murfin, S.; Wilkes, R.; Whittaker, M.; Watkin, D. J.; Fleet, G. W. J. *J. Comb. Chem.* **2004**, *6*, 230−238.

(5) (a) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491−514. (b) Chakraborty, T. K.; Jayaprakash, S.; Ghosh, S. *Comb. Chem. High Throughput Screening* **2002**, *5*, 373−387. (c) Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230−253. (d) Risseeuw, M. D. P.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. *Tetrahedron: Asymmetry* **2007**, *18*, 2001−2010. (e) Jiménez Blanco, J. L.; Ortega-Caballero, F.; Ortiz Mellet, C.; García Fernández, J. M. *Beilstein J. Org. Chem.* **2010**, *6*, No. 20. (f) Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis* **2010**, *2010*, 3217−3242. (g) Rjabovs, V.; Turks, M. *Tetrahedron* **2013**, *69*, 10693−10710. (h) Risseeuw, M.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. *Amino Acids* **2013**, *45*, 613−689. (i) Kiss, L.; Fülöp, F. *Chem. Rev.* **2014**, *114*, 1116−1169.

(6) Knapp, S.; Shieh, W.-C.; Jaramillo, C.; Trilles, R. V.; Nandan, S. R. *J. Org. Chem.* **1994**, *59*, 946−948.

(7) Fox, J. J.; Kuwada, Y.; Watanabe, K. A. *Tetrahedron Lett.* **1968**, *9*, 6029−6032.

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485

(8) Lichtenthaler, F. W.; Morino, T.; Menzel, H. M. *Tetrahedron Lett.* **1975**, *16*, 665−668.
(9) (a) Nakajima, M.; Itoi, K.; Takamatsu, Y.; Kinoshita, T.; Okazaki, T.; Kawakubo, K.; Shindo, M.; Honma, T.; Tohjigamori, M.; Haneishi, T. *J. Antibiot.* **1991**, *44*, 293−300. (b) Haruyama, H.; Takayama, T.; Kinoshita, T.; Kondo, M.; Nakajima, M.; Haneishi, T. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1637−1640.
(10) (a) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156−10167. (b) Chakraborty, T. K.; Jayaprakash, S.; Diwan, P. V.; Nagaraj, R.; Jampani, S. R. B.; Kunwar, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 12962−12963.
(11) Graf von Roedern, E.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 687−689.
(12) (a) van Well, R. M.; Overkleeft, H. S.; van der Marel, G. A.; Bruss, D.; Thibault, G.; de Groot, P. G.; van Boom, J. H.; Overhand, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 331−334. (b) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S.; Sarma, J. J. A. R. P.; Ravikanth, V.; Diwan, P. V.; Nagaraj, R.; Kunwar, A. C. *J. Org. Chem.* **2000**, *65*, 6441−6457.
(13) Gruner, S. A. W.; Kéri, G.; Schwab, R.; Venetianer, A.; Kessler, H. *Org. Lett.* **2001**, *3*, 3723−3725.
(14) Knijnenburg, A. D.; Spalburg, E.; de Neeling, A. J.; Mars-Groenendijk, R. H.; Noort, D.; Grotenbreg, G. M.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *Helv. Chim. Acta* **2012**, *95*, 2544−2561.
(15) (a) Claridge, T. D. W.; Long, D. D.; Hungerford, N. L.; Aplin, R. T.; Smith, M. D.; Marquess, D. G.; Fleet, G. W. J. *Tetrahedron Lett.* **1999**, *40*, 2199−2202. (b) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189−8196. (c) Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem. - Eur. J.* **2002**, *8*, 4365−4376. (d) Suhara, Y.; Yamaguchi, Y.; Collins, B.; Schnaar, R. L.; Yanagishita, M.; Hildreth, J. E. K.; Shimada, I.; Ichikawa, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1999−2013. (e) Chandrasekhar, S.; Reddy, M. S.; Jagadeesh, B.; Prabhakar, A.; Ramana Rao, M. H. V.; Jagannadh, B. *J. Am. Chem. Soc.* **2004**, *126*, 13586−13587. (f) Chakraborty, T. K.; Koley, D.; Ravi, R.; Kunwar, A. C. *Org. Biomol. Chem.* **2007**, *5*, 3713−3716. (g) Siriwardena, A.; Pulukuri, K. K.; Kandiyal, P. S.; Roy, S.; Bande, O.; Ghosh, S.; Garcia Fernandez, J. M.; Ariel Martin, F.; Ghigo, J.-M.; Beloin, C.; Ito, K.; Woods, R. J.; Ampapathi, R. S.; Chakraborty, T. K. *Angew. Chem., Int. Ed.* **2013**, *52*, 10221−10226. (h) Feher-Voelger, A.; Borges-Gonzalez, J.; Carrillo, R.; Morales, E. Q.; Gonzalez-Platas, J.; Martin, T. *Chem. - Eur. J.* **2014**, *20*, 4007−4022.
(16) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(17) (a) Wessel, H. P.; Mitchell, C.; Lobato, C. M.; Schmid, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2712−2713. (b) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575−1578. (c) Sabesan, S. *Tetrahedron Lett.* **1997**, *38*, 3127−3130. (d) Gregar, T. Q.; Gervay-Hague, J. *J. Org. Chem.* **2004**, *69*, 1001−1009.
(18) Sicherl, F.; Wittmann, V. *Angew. Chem., Int. Ed.* **2005**, *44*, 2096−2099.
(19) Tor, Y.; Hermann, T.; Westhof, E. *Chem. Biol.* **1998**, *5*, R277−R283.
(20) (a) Meredith, H. R.; Srimani, J. K.; Lee, A. J.; Lopatkin, A. J.; You, L. *Nat. Chem. Biol.* **2015**, *11*, 182−188. (b) Becker, B.; Cooper, M. A. *ACS Chem. Biol.* **2013**, *8*, 105−115. (c) Lewis, K. *Nat. Rev. Drug Discovery* **2013**, *12*, 371−387.
(21) Grotenbreg, G. M.; Kronemeijer, M.; Timmer, M. S. M.; El Oualid, F.; van Well, R. M.; Verdoes, M.; Spalburg, E.; vanHooft, P. A. V.; de Neeling, A. J.; Noort, D.; van Boom, J. H.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *J. Org. Chem.* **2004**, *69*, 7851−7859.
(22) For easy comparison, we use the numbering of the skeletal carbons of compounds according to that of the parent compound 3,5-diaminoribose throughout this article.
(23) Lundquist, J. T., IV; Pelletier, J. C. *Org. Lett.* **2002**, *4*, 3219−3221.
(24) Dangerfield, E. M.; Plunkett, C. H.; Win-Mason, A. L.; Stocker, B. L.; Timmer, M. S. M. *J. Org. Chem.* **2010**, *75*, 5470−5477.
(25) (a) Gabriel, S. *Ber. Dtsch. Chem. Ges.* **1887**, *20*, 2224−2236. (b) Ing, H. R.; Manske, R. H. F. *J. Chem. Soc.* **1926**, *129*, 2348−2351.
(26) (a) Bochkov, A. F.; Kochetkov, N. K. *Carbohydr. Res.* **1975**, *39*, 355−357. (b) de las Heras, F. G.; Fernandez-Resa, P. *J. Chem. Soc., Perkin Trans. 1* **1982**, 903−907. (c) Utimoto, K.; Wakabayashi, Y.; Horiie, T.; Inoue, M.; Shishiyama, Y.; Obayashi, M.; Nozaki, H. *Tetrahedron* **1983**, *39*, 967−973. (d) Kochetkov, N. K. *Tetrahedron* **1987**, *43*, 2389−2436. (e) Vogel, C.; Torres, G. M.; Reinke, H.; Michalik, D.; Voss, A. *Carbohydr. Res.* **2007**, *342*, 520−528. (f) Worch, M.; Wittmann, V. *Carbohydr. Res.* **2008**, *343*, 2118−2129.
(27) Liang, C. W.; Jung Kim, M.; Shin Jeong, L.; Woo Chun, M. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 2039−2048.
(28) Bolon, P. J.; Sells, T. B.; Nuesca, Z. M.; Purdy, D. F.; Nair, V. *Tetrahedron* **1994**, *50*, 7747−7764.
(29) (a) Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2350−2353. (b) Wittmann, V.; Datta, A. K.; Koeller, K. M.; Wong, C.-H. *Chem. - Eur. J.* **2000**, *6*, 162−171. (c) Hünig, S.; Müller, H. R.; Thier, W. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 271−280.
(30) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139−164.
(31) Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093−2096.
(32) Fuji, K.; Nakano, S.; Fujita, E. *Synthesis* **1975**, *1975*, 276−277.
(33) Wellings, D. A.; Atherton, E. *Methods Enzymol.* **1997**, *289*, 44−67.
(34) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787−3790.
(35) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (b) Carpino, L. A.; El-Faham, A.; Minor, C.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203.
(36) McCroskey, M.; Groppi, V. E.; Pearson, J. D. *Anal. Biochem.* **1987**, *163*, 427−432.
(37) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

DOI: 10.1021/acs.joc.5b01049
J. Org. Chem. 2015, 80, 7477−7485