

A New Phenoxyacetate-Based Linker System for the Solid-Phase Synthesis of Oligosaccharides

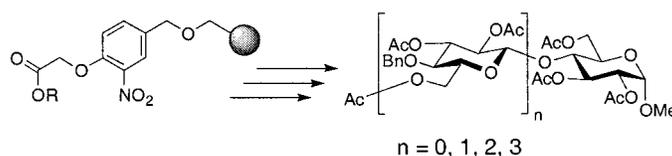
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ABSTRACT



A novel linker system has been designed, and its first application to solid-phase oligosaccharide synthesis is described. The use of the highly reactive *o*-nitro-phenoxyacetate linker allows a fast and quantitative cleavage using mild basic conditions. This method combined with the trichloroacetimidate glycosylation exhibits highly promising results as demonstrated for the synthesis of tetrasaccharide **1** ($n = 3$) containing glucose $\beta(1 \rightarrow 4)$ and $\beta(1 \rightarrow 6)$ linkages.

The solid-phase synthesis of oligosaccharides has gained considerable interest during the last years¹ as a result of its possible advantages over conventional technologies, and many different linker systems have been developed.² To employ the powerful trichloroacetimidate glycosylation method,³ temporary protective groups and the linker moiety have to be designed to be stable in the presence of catalytic

amounts of Lewis acid and to permit orthogonal cleavage under mild conditions. Most of the commonly used linker types in synthetic organic chemistry or peptide chemistry⁴ do not fulfill this demand. Therefore, the design of advanced protective groups and new efficient linker systems for the solid-phase synthesis of oligosaccharides remains a challenging task.

We present here our results concerning the preparation and first application of a new *o*-nitro-substituted phenoxyacetate linker system on Merrifield resin. Since it is known from solution-phase chemistry that an unsubstituted phenoxyacetate protecting group can be easily cleaved using methylamine, we chose the *o*-nitro-substituted derivative, which as a result of its electron-withdrawing effect should permit a particularly short reaction time for cleavage from the solid support.⁵ The system proved to meet all the requirements given above, leading to target tetrasaccharide molecule **1** in an excellent yield (Scheme 1). To the best of our knowledge the structural motif **1** was until now only

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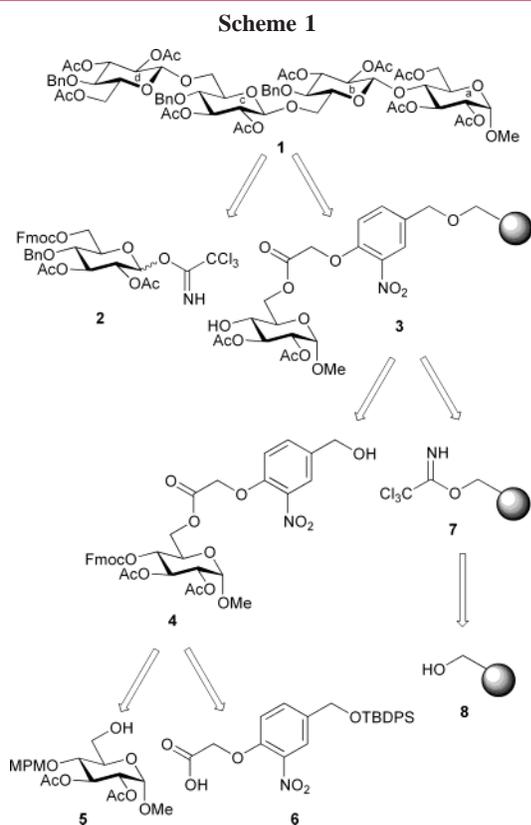
(2) (a) Ester type linker: Roussel, F.; Knerr, L.; Grathwohl, M.; Schmidt, R. R. *Org. Lett.* **2000**, *2*, 3043–3046. Zhu, T.; Boons, G. J. *J. Am. Chem. Soc.* **2000**, *122*, 10222–10223. (b) Silyl ether type linker: Doi, T.; Sugiki, M.; Yamada, H.; Takahashi, T.; Porco, J. A., Jr. *Tetrahedron Lett.* **1999**, *40*, 2141–2144. Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307–1309. (c) Thioether type linker: Rademann, J.; Geyer, A.; Schmidt, R. R. *Angew. Chem.* **1998**, *110*, 1309–1313; *Angew. Chem., Int. Ed.* **1998**, *37*, 1241–1245. Yan, L.; Taylor, C. M.; Goodnow, R., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953–6954. (d) Benzyl ether type linker: Mehta, S.; Whitfield, D. M. *Tetrahedron Lett.* **1998**, *39*, 5907–5910. (e) Sulfone linker: Hunt, J. A.; Roush, W. R. *J. Am. Chem. Soc.* **1996**, *118*, 9998–9999. (f) Wang type linker: Manabe, S.; Ito, Y.; Ogawa, T. *Synlett* **1998**, 628–630. (g) Metathesis cleavable linker: Knerr, L.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 2803–2808. Andrade, R. B.; Plante, O. J.; Melean, L.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811–1814. (h) Selenium based linker: Nicolaou, K. C.; Mitchell, H. J.; Fylaktakidou, K. C.; Suzuki, H.; Rodríguez, R. M. *Angew. Chem.* **2000**, *112*, 1131–1135; *Angew. Chem., Int. Ed.* **2000**, *39*, 1089–1093.

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(4) For a recent review on linkers, see: James, I. W. *Tetrahedron* **1999**, *55*, 4855–4946.

(5) For the use of this type of system in a reverse manner in oligosaccharide synthesis on Wang resin, see: Manabe, S.; Nakahara, Y.; Ito, Y. *Synlett* **2000**, 9, 1241–1244.

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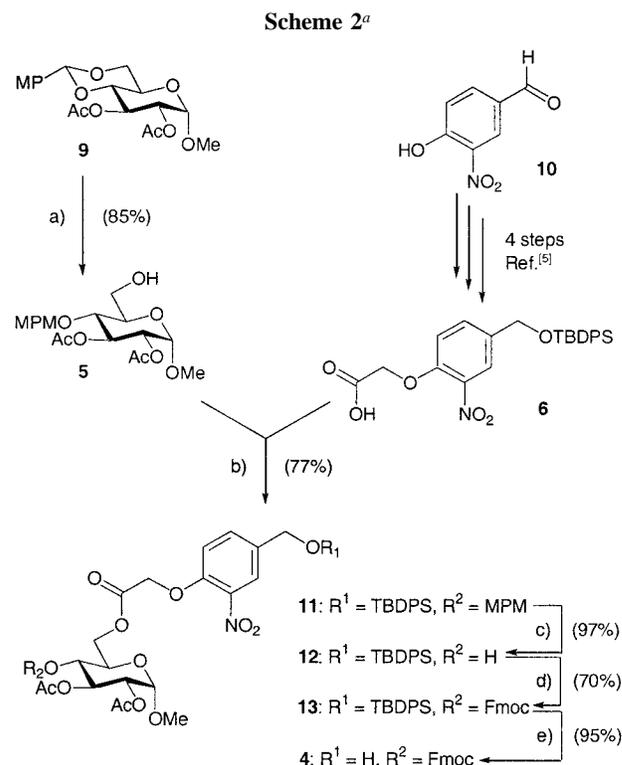


synthesized by an enzymatic approach under regioselective transglycosylation conditions in low yield.⁶

Retrosynthetic analysis of **1** led to the polymer-bound linker **3** and to the 9-fluorenylmethoxycarbonyl (Fmoc) group bearing *O*-glucosyl trichloroacetimidate **2**.⁷ Recently, we reported the efficient preparation of *O*-glycosyl trichloroacetimidates bearing Fmoc-protected hydroxy groups and their use as glycosyl donors both in solution and on solid phase.⁸ Other types of Fmoc-containing glycosyl donors have been recently also reported.^{9,10} Resin **3** led to the required building blocks **4–6** as well as to the activated trichloroacetimidate Merrifield resin **7**,¹¹ which was synthesized starting from commercially available hydroxymethyl-substituted Merrifield resin **8**. We describe here the first application of polymer reagent **7** in an acid-catalyzed benzyl ether synthesis. Compound **7** and all derivatives thereof exhibited higher stability against acids, as required for the solid-phase oligosaccharide synthesis, than the corresponding Wang resin.¹²

The synthesis of **1** begins with suitably protected known precursor **9**,¹³ which is converted under reductive reaction

conditions into the corresponding primary alcohol **5** by a highly regioselective *p*-methoxy-benzylidene ring opening (Scheme 2).



^a (a) 0.1 equiv Bu₂BOTf, BH₃·THF, CH₂Cl₂, 0 °C. (b) DCC, DMAP, CH₂Cl₂, rt. (c) DDQ, CH₂Cl₂/H₂O, rt. (d) 2 equiv FmocCl, pyridine, rt. (e) HF·pyridine, THF, rt.

Starting from commercially available 4-hydroxy-3-nitrobenzaldehyde **10** the *o*-nitro-substituted phenoxyacetyl linker molecule **6** was prepared following a four-step literature procedure.⁵ Ester formation between primary alcohol **5** and carboxylic acid **6** was achieved by treatment with a catalytic amount of DMAP (0.1 equiv) and with DCC (2.0 equiv) as condensing agent, affording fully protected **11**. Cleavage of the MPM ether moiety of **11** was performed in the presence of DDQ (1.5 equiv) in a mixture of CH₂Cl₂/H₂O to obtain **12** in an excellent yield. Because of its high stability toward Lewis acid as well as its convenient cleavage under weak basic conditions, which can be readily followed by UV, for temporary protection the Fmoc group was employed. In our

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(9) For Fmoc-containing phosphoramidites, see: Freese, S. J.; Vann, W. F. *Carbohydr. Res.* **1996**, *281*, 313–319.

(10) For Fmoc-containing thioglycoside donors, see: (a) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449–450. (b) Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem.* **1998**, *110*, 1636–1638; *Angew. Chem., Int. Ed.* **1998**, *37*, 1559–1561. (c) Zhu, T.; Boons, G.-J. *Tetrahedron: Asymm.* **2000**, *11*, 199–205.

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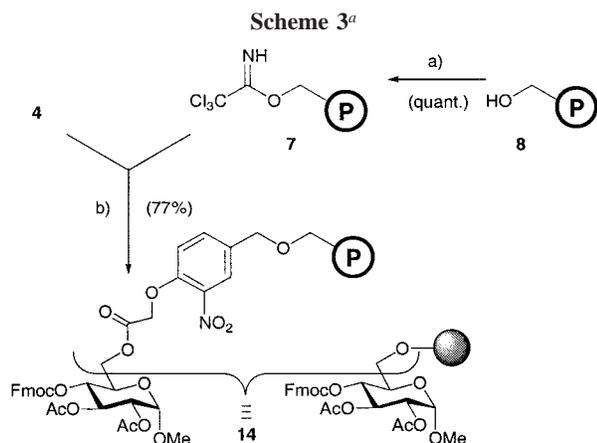
(12) For Wang trichloroacetimidate resin, see: Hanessian, S.; Xie, F. *Tetrahedron Lett.* **1998**, *39*, 733–736.

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(15) **Preparation of Trichloroacetimidate Resin 7.** After swelling of dry Merrifield resin **8** (10.0 g, 14.6 mmol) in CH₂Cl₂ (100 mL), trichloroacetonitrile (15 mL, 10 equiv) was added. The resulting suspension was cooled under argon to 0 °C and shaken for 10 min. DBU (0.44 mL, 0.2 equiv) was added, and the resulting mixture was shaken for 40 min under an inert gas atmosphere at 0 °C. The resin was rinsed off, switching three times between CH₂Cl₂ and THF (each 100 mL), and dried under high vacuum to afford resin **7** (12.9 g, 14.6 mmol, quant).

hands this group has emerged as a very suitable protecting group for both solution- and solid-phase synthesis.⁸ Installation of the Fmoc group to **12** using pyridine and FmocCl (\rightarrow **13**) was followed by desilylation using 5 equiv of HF-py in THF¹⁴ to afford linker **4**, ready for loading onto the resin. Attachment of linker **4** to the hydroxymethyl-substituted Merrifield resin **8** was achieved via formation of trichloroacetimidate resin **7** and its activation with a catalytic amount of TMSOTf (0.3 equiv) at 0 °C (Scheme 3).



^a (a) 10 equiv CCl_3CN , CH_2Cl_2 , 0 °C. (b) (i) 0.3 equiv TMSOTf, CH_2Cl_2 , 0 °C. (ii) MeOH.

It is to be noted that conversion of **8** to **7** proceeds smoothly and very quickly in a quantitative manner. The reaction can be readily performed up to a 10-g scale, and the resulting activated trichloroacetimidate polymer reagent **7** showed a high stability and was storable for at least 6 months.¹⁵ After linker attachment affording resin **14** the remaining activated benzyl functions were quenched by direct addition of methanol. Loading of resin **14** was determined by a very fast and clean preparative cleavage of **15** by excess MeNH_2 (10 min) followed by acetylation to be 0.213 mmol/g (Scheme 4).¹⁶ Under these conditions the linker was selectively cleaved without affecting any *O*-acetyl groups; loading was also determined by recycling unreacted linker molecule **4** from the washing solution.

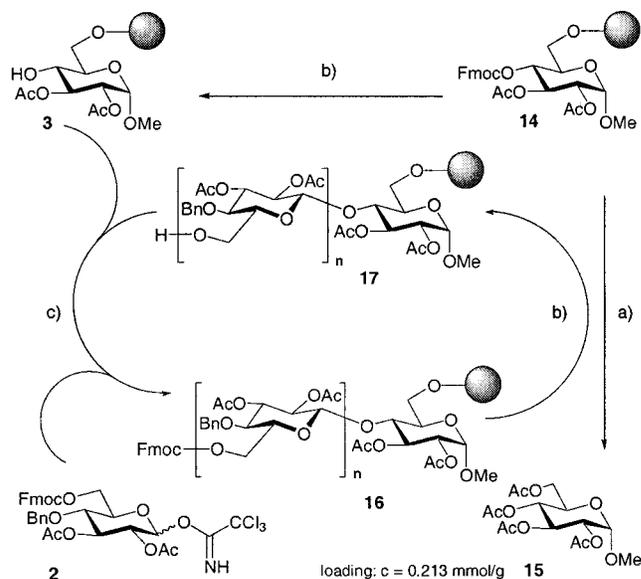
Generation of polymer-bound acceptor **3** was performed by treatment with a mixture of dichloromethane and triethy-

(16) **General Procedure for Cleavage.** Dry resin was swollen in CH_2Cl_2 (10 mL/g resin), and the resulting suspension was shaken for 10 min under an inert gas atmosphere. A solution of ~ 8 M MeNH_2 in EtOH (~ 100 equiv) was added, and the resulting mixture was shaken for 10 min under argon. The resin was rinsed off, switching three times between CH_2Cl_2 and THF. The evaporated filtrates were treated by a mixture of acetic anhydride and pyridine (1:1, 20 mL/g) for 2 h. The resulting residues were purified by flash chromatography.

(17) **General Procedure for Deprotection.** Dry resin was swollen in a mixture of $\text{CH}_2\text{Cl}_2/\text{NEt}_3$ (4:1), and the resulting suspension was shaken for 4 h. The resin was treated as described above and dried under high vacuum.

(18) **General Procedure for Glycosylation.** Dry acceptor loaded resin was directly swollen in a CH_2Cl_2 solution (15 mL/g resin) containing donor **2** (3 equiv) and 4 Å molecular sieves. The resulting suspension was cooled under argon to 0 °C and shaken for 10 min. A solution of a freshly prepared 0.5 M TMSOTf solution in CH_2Cl_2 (0.3 equiv) was added, and the resulting mixture was shaken for 1 h under an inert gas atmosphere. The resin was treated as described above.

Scheme 4^a



^a (a) (i) MeNH_2 , CH_2Cl_2 , rt. (ii) Ac_2O /pyridine, rt. (b) NEt_3 , CH_2Cl_2 , rt. (c) 0.3 equiv TMSOTf, 4 Å, CH_2Cl_2 , 0 °C.

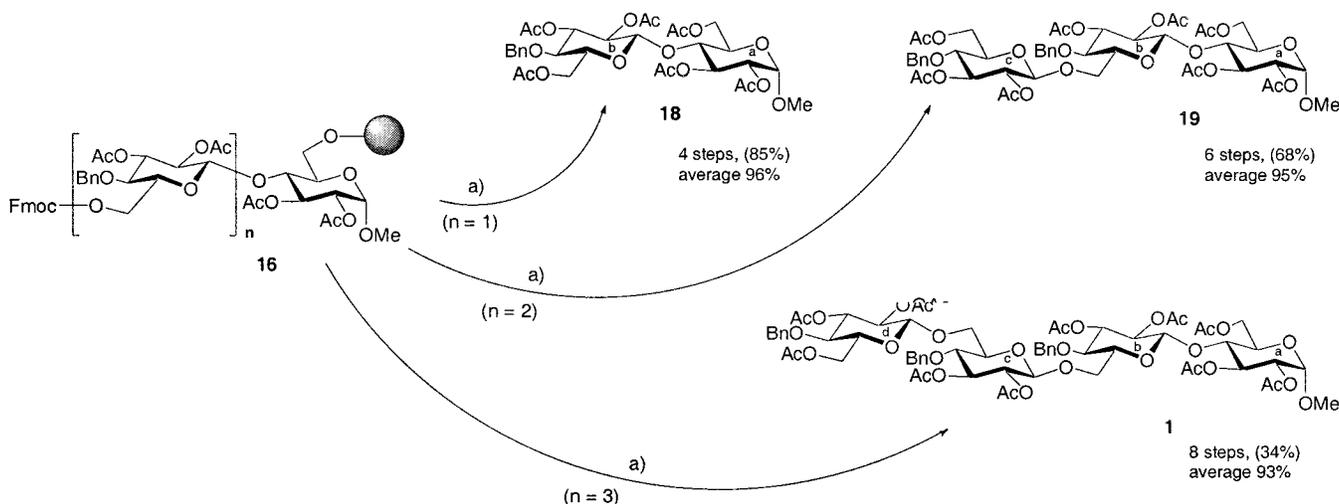
lamine until the generated UV spot completely disappeared.¹⁷ Solid-phase glycosylation with the Fmoc-bearing *O*-glucosyl trichloroacetimidate **2** (3.0 equiv) at 0 °C and repetition of the described sequence in a cyclic manner afforded resin-bound oligosaccharides **16** ($n = 1-3$) and **17** ($n = 1, 2$).¹⁸ All glycosylations were performed only once with the exception of the first one, which required repetition in order to reach a complete $\beta(1 \rightarrow 4)$ linkage.¹⁹

The described solid-phase synthesis cycle was performed three times. After each run preparative cleavage of the polymer-bound structures **16** ($n = 1-3$) using the conditions described above¹⁶ furnished the corresponding oligosaccharides **18**, **19**, and **1** in excellent yields (Scheme 5). Cellobiose derivative **18**²⁰ was isolated in 85% overall yield over four steps starting from **14** (96% per step). Up to the tetrameric stage no significant drop in terms of efficiency of this new

(19) Solid-phase glycosylation to resin bound disaccharide **16** ($n = 1$) was repeated once under the same conditions.

(20) **Analytical Data of Compound 18.** MALDI-TOF (DHB/THF): m/z calcd M ($\text{C}_{32}\text{H}_{42}\text{O}_{17}$) 698.67; (M + Na)⁺ 721.66; (M + K)⁺ 737.77; found 721.9, 737.9. ¹H NMR (600 MHz, CDCl_3): δ 2.06 (m, 6 COCH_3), 3.36 (s, OCH_3), 3.55 (m, 5b-*H*), 3.65 (t, ³ $J = 3.3$ Hz, 4b-*H*), 3.71 (t, ³ $J = 3.2$ Hz, 4a-*H*), 3.87 (m, 5a-*H*), 4.13 (dd, $J_{6,6'} = 12.0$ Hz, $J_{5,6} = 4.8$ Hz, 6a-*H*), 4.27 (2d, ³ $J = 3.7$ Hz, 6'b-*H*, 6b-*H*), 4.49 (d, $J_{1,2} = 7.9$ Hz, 1b-*H*), 4.50 (d, ³ $J = 3.0$ Hz, 6'a-*H*), 4.53–4.59 (m, CH_2Ph), 4.80 (dd, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 10.2$ Hz, 2a-*H*), 4.84 (m, 2b-*H*), 4.85 (d, $J_{1,2} = 3.7$ Hz, 1a-*H*), 5.19 (t, ³ $J = 9.2$ Hz, 3b-*H*), 5.43 (t, ³ $J = 9.7$ Hz, 3a-*H*), 7.21–7.31 (m, *Ar*). ¹³C NMR (150.9 MHz, CDCl_3): $\delta = 62.2$ (C-6a), 63.1 (C-6b), 65.4 (C-5a), 70.1 (C-3a), 71.3 (C-2a), 72.5 (C-2b), 73.3 (C-5b), 75.5 (C-4b), 75.7 (C-3b), 77.0 (C-4a), 97.1 (C-1a), 101.1 (C-1b).

(21) **Analytical Data of Compound 19.** MALDI-TOF (DHB/THF): m/z calcd M ($\text{C}_{49}\text{H}_{62}\text{O}_{24}$) 1035.00; (M + Na)⁺ 1057.99; (M + K)⁺ 1074.10; found 1057.3, 1073.3. ¹H NMR (600 MHz, CDCl_3): δ 2.03 (m, 8 COCH_3), 3.36 (s, OCH_3), 3.40 (m, 5b-*H*), 3.55 (m, 5c-*H*), 3.62 (m, 4b-*H*, 4c-*H*), 3.66 (m, 6b-*H*), 3.69 (m, 4a-*H*), 3.76 (d, ³ $J = 7.3$ Hz, 5a-*H*), 4.10 (m, 6'b-*H*), 4.13 (d, ³ $J = 5.0$ Hz, 6a-*H*), 4.17 (dd, $J_{6,6'} = 12.0$ Hz, $J_{5,6} = 4.6$ Hz, 6c-*H*), 4.34 (d, ² $J = 2.2$ Hz, 6'c-*H*), 4.44 (d, $J_{1,2} = 7.9$ Hz, 1b-*H*), 4.48 (d, $J_{1,2} = 7.9$ Hz, 1c-*H*), 4.49 (d, ² $J = 2.1$ Hz, 6'a-*H*), 4.53–4.59 (m, 2 CH_2Ph), 4.80 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 6.2$ Hz, 2b-*H*), 4.81 (m, 2a-*H*),

Scheme 5^a

^a (a) (i) MeNH₂, CH₂Cl₂, rt. (ii) Ac₂O/pyridine, rt.

method was observed. Thus, trisaccharide **19**²¹ (68% overall over six steps, average 95% per step) and tetrasaccharide **1**²² (34% overall over eight steps, average 93% per step) were isolated in similar remarkable yields.

4.86 (d, $J_{1,2} = 3.5$ Hz, 1a-*H*), 4.89 (m, 2c-*H*), 5.17 (t, $^3J = 9.3$ Hz, 3b-*H*), 5.23 (t, $^3J = 9.4$ Hz, 3c-*H*), 5.42 (t, $^3J = 9.7$ Hz, 3a-*H*), 7.22–7.32 (m, *Ar*). ¹³C NMR (150.9 MHz, CDCl₃): δ 62.2 (C-6a), 62.8 (C-6c), 68.1 (C-6b), 70.0 (C-3a), 71.4 (C-2a), 72.3 (C-2c), 72.7 (C-2b), 73.4 (C-5c), 74.7 (C-5b), 75.6 (C-3c), 75.7 (C-3b), 76.0 (C-4b, C-4c), 77.0 (C-4a), 97.0 (C-1a), 100.9 (C-1c), 101.3 (C-1b).

(22) **Analytical Data of Compound 1.** MALDI-TOF (DHB/THF): *m/z* calcd M (C₆₆H₈₂O₃₁) 1371.34; (M + Na)⁺ 1394.33; (M + K)⁺ 1410.44; found 1393.7, 1410.3. ¹H NMR (600 MHz, CDCl₃): δ 2.02 (m, 11 COCH₃), 3.35 (s, OCH₃), 3.48 (m, 4c-*H*), 3.50 (m, 5b-*H*), 3.51 (m, 5c-*H*), 3.58 (m, 5d-*H*), 3.64 (d, $J = 2.3$ Hz, 6c-*H*), 3.65 (d, $J = 2.4$ Hz, 6b-*H*), 3.67 (m, 4b-*H*), 3.68 (m, 4d-*H*), 3.72 (d, $^3J = 9.6$ Hz, 4a-*H*), 3.85 (m, 5a-*H*), 4.03 (d, $^2J = 10.8$ Hz, 6'b-*H*), 4.12 (t, 6a-*H*), 4.20 (m, 6'c-*H*, 6d-*H*), 4.37 (dd, $J_{6,6'} = 12.0$ Hz, $J_{5,6'} = 2.1$ Hz, 6'd-*H*), 4.46 (m, 1c-*H*, 6'a-*H*), 4.49 (d, $J_{1,2} = 8.0$ Hz, 1b-*H*), 4.52–4.59 (m, 3 CH₂Ph), 4.58 (m, 1d-*H*), 4.81 (d, $J_{1,2} = 3.2$ Hz, 1a-*H*), 4.82 (m, 2b-*H*), 4.84 (m, 2c-*H*), 4.86 (m, 2a-*H*), 4.89 (m, 2d-*H*), 5.18 (m, 3b-*H*), 5.20 (m, 3c-*H*), 5.21 (m, 3d-*H*), 5.40 (t, $^3J = 9.7$ Hz, 3a-*H*), 7.22–7.35 (m, *Ar*). ¹³C NMR (150.9 MHz, CDCl₃): δ 62.3 (C-6a), 62.9 (C-6d), 67.6 (C-6c), 68.5 (C-6b, C-5a), 70.1 (C-3a), 71.2 (C-2a), 72.1 (C-2d), 72.4 (C-2c), 72.9 (C-2b), 73.6 (C-5d), 74.2 (C-5b), 75.3 (C-5c, C-3d), 75.6 (C-3c), 75.8 (C-3, C-4d), 75.9 (C-4b), 76.7 (C-4a, C-4c), 96.9 (C-1a), 100.8 (C-1c), 101.1 (C-1b), 101.4 (C-1d).

In summary, we have presented the synthesis of a new highly reactive linker system and its first successful application to solid-phase oligosaccharide synthesis. This novel *o*-nitro-phenoxyacetate linker proved to be completely stable under glycosylation and Fmoc removal conditions and could be cleaved very quickly at the end of the synthesis using only volatile reagents. Thus, Fmoc as the temporary hydroxy protecting group of *O*-glycosyl trichloroacetimidates was found to be a very powerful combination leading to tetrasaccharide **1** in an excellent overall yield. Currently we are developing the application of this new linker system toward higher oligomers and more complex carbohydrate structures.

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