[5] a) For a "traceless linker" for isoquinolines, see B. A. Lorsbach, J. T. Bagdanoff, R. B. Miller, M. J. Kurth, *J. Org. Chem*. **1998**, *63*, 2244 – 2250; b) for a "linker" for amines, see X. Ouyang, R. W. Armstrong, M. M. Murphy, *J. Org. Chem*. **1998**, *63*, 1027 – 1032; c) for a photolabile benzyl sulfide linker, see F. W. Forman, I. Sucholeiki, *J. Org. Chem*. **1995**, *60*, 523 – 528; d) for a "traceless linker" for carboxylic acids, see P. Garibay, J. Nielsen, T. Hoeg-Jensen, *Tetrahedron Lett.* **1998**, *39*, 2207 – 2210; e) P. Conti, D. Demont, J. Cals, H. C. J. Ottenheijm, D. Leysen, *Tetrahedron Lett.* **1997**, *38*, 2915 – 2918; f) C. R. Johnson, B. Zhang, *Tetrahedron Lett.* **1995**, *36*, 9253 – 9256; g) for the use of sulfones as linkers, see L. M. Gayo, M. J. Suto, *Tetrahedron Lett.* **1997**, *38*, 211 – 214; h) for the use of an aliphatic C-H "traceless" linker, see X. Zhao, K. W. Jung, K. D. Janda, *Tetrahedron Lett.* **1997**, *38*, 977 – 980; *Tetrahedron Lett.* **1997**, *38*, 6645 – 6652; K. W. Jung, X. Zhao, K. D. Janda, *Tetrahedron Lett.* **1996**, *37*, 6491 – 6494; i) for further linkers, see **I.** Hughes, *Tetrahedron Lett.* **1996**, *37*, 7595 – 7598; j) R. Brown, D. C. Rees, Z. Rankovic, J. R. Morphy, *J. Am. Chem. Soc*. **1997**, *119*, 3288 – 3295; k) X. Zhao, K. D. Janda, *Tetrahedron Lett*. **1997**, *38*, 5437 – 5440; l) S. W. Kim, S. Y. Ahn, J. S. Koh, J. H. Lee, S. R. Ro, H. Y. Cho, *Tetrahedron Lett.* **1997**, *38*, 4603 – 4606; m) A. K. Szardenings, T. Burkoth, H. H. Lu, D. W. Tien, D. A. Campbell, *Tetrahedron* **1997**, *53*, 6573 – 6593; n) M. R. Gowravaram, M. A. Gallop, *Tetrahedron Lett*. **1997**, *38*, 6973 – 6976; o) A. D. Piscopio, J. F. Miller, K. Koch, *Tetrahedron Lett.* **1997**, *38*, 7143 – 7146; p) F. E. K. Kroll, R. Morphy, D. Rees, D. Gani, *Tetrahedron Lett.* **1997**, *38*, 8573 – 8576; q) for cleavage by metathesis, see J. U. Peters, S. Blechert, *Synlett* **1997**, 348 – 350; r) for an enzyme-labile linker, see B. Sauerbrei, V. Jungmann, H. Waldmann, *Angew. Chem*. **1998**, *110*, 1187 – 1190. *Angew. Chem. Int. Ed.* **1998**, *37*, 1143 – 1146.

[6] a) A. Engel in *Methoden Org. Chem. (Houben-Weyl*), 4th ed. 1952 – , Vol. E 16a, **1990**, pp. 1182 – 1226; b) K. C. Nicolaou, C. N. C. Boddy, S. Natarajan, T.-Y. Yue, H. Li, S. Bräse, J. M. Ramanjulu, *J. Am. Chem. Soc*. **1997**, *119*, 3412 – 3422, and references therein; c) S. Bräse, J. Köbberling, D. Enders, R. Lazny, J. Poplawski, *Tetrahedron Lett.*, submitted; d) S. Bräse, J. Köbberling, D. Enders, M. Wang, R. Lazny, *Tetrahedron Lett.*, submitted.

[7] a) P. J. Das, S. Khound, *Tetrahedron* **1994**, *50*, 9107 – 9112; b) J. C. Nelson, J. K. Young, J. S. Moore, *J. Org. Chem*. **1996**, *61*, 8160 – 8168.

[8] The modified resins were characterized by C,H,N analysis.

[9] The resins prepared, with the exception of the examples that contain iodine, are stable against light.

[10] S. Bräse, A. de Meijere in *Metal-catalyzed Cross-Coupling Reactions* (Eds.: F. Diederich, P. J. Stang), WILEY-VCH, Weinheim, **1998**, 99 – 167, and references therein.

[11] a) For examples on a solid support, see V. Yedida, C. C. Leznoff, *Can. J. Chem*. **1980**, *58*, 1144 – 1150; b) L. F. Tietze, T. Hippe, A. Steinmetz, *Synlett* **1996**, 1043 – 1044; c) thermal Diels – Alder reactions on a cinnamonic acid derivative need higher temperature.

[12] a) H. C. Kolb, M. S. VanNieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483 – 2547; b) H. Han, K. D. Janda, *Angew. Chem*. **1997**, *109*, 1835 – 1837; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1731 – 1733.

[13] L. F. Tietze, T. Hippe, A. Steinmetz, *Chem. Commun*. **1998**, 793 – 794, and references therein.

[14] a) N. Kornblum, *Org. React*. **1944**, *2*, 262; b) N. Kornblum, A. E. Kelly, G. D. Cooper, *J. Am. Chem. Soc*. **1952**, *74*, 3074 – 3076, and references therein.

[15] This kind of effect in which solid-phase chemistry is different to the corresponding reactions in the liquid phase is known.[1] Therefore, observations in either system are not always transferable. In this case a Meerwein reduction of the regenerated diazonium salt seems to be favored on the solid phase since the resin might enhance a radical mechanism relative to an ionic pathway.

[16] After longer reaction times (> 10 min) or higher reaction temperatures (> 50 °C) polymers of the solvent could be detected, which were removed by solid-phase extraction. Literature known compounds are identified by their spectroscopic data and by comparison with authentic samples. All new compounds gave correct spectroscopic data (IR, MS, NMR).

[17] The purity of the products are reproducibly excellent. In the case of lower yields, repetitive cleavage results in more material.

[18] S. Bräse, D. Enders, J. Köbberling, unpublished results.

[19] N. Satyamurthy, J. R. Barrio, *J. Org. Chem*. **1983**, *48*, 4394 – 4396.

Quantitative Monitoring of Solid-Phase Synthesis Using Gated Decoupling ^{13}C NMR Spectroscopy with a ^{13}C-Enriched Protecting Group and an Internal Standard in the Synthesis of Sialyl Lewis^x Tetrasaccharide**

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Polymer-supported organic synthesis is gaining increasing attention in connection to combinatorial chemistry. Despite the recent success of polymer-supported oligosaccharide synthesis, methods for the the qualitative and quantitative nondestructive monitoring of the reaction have not yet been developed. Among such monitoring approaches, those based on NMR spectroscopy[1] are perhaps the most useful. We are particularly interested in using ^{13}C NMR spectroscopy in this context since it can be performed with conventional high-field NMR spectrometers.[2] The ordinary method is, however, not realistic due to the low signal intensities and the lack of quantitative information, especially when small amounts of compounds are being synthesized for screening purposes. To overcome this problem, Look et al. reported the use of ^{13}C-enriched synthetic blocks in polymer-supported organic synthesis as a potential application to combinatorial chemistry.[2d] This method is indeed important; however, the incorporation of ^{13}C nuclei into a synthon will determine the practicality of the approach. The nuclear Overhauser effect (NOE) from directly attached proton(s) may also affect the integration. To demonstrate a successful application of the method to oligosaccharide synthesis, we have used a ^{13}C-enriched protecting group for the glycosylation reagents together with a ^{13}C-enriched internal standard. Also, the gated decoupling ^{13}C NMR spectroscopy[3] is performed in the presence of a relaxation agent to avoid complications arising from NOEs in the quantitative monitoring of the reaction. Hence the reaction progress can be monitored by integration of the ^{13}C NMR signal of the protecting group and comparing with that of the internal integral standard. Using this strategy, we report herein the first chemical solid-phase synthesis of sialyl Lewis^x tetrasaccharide (sLe^x), which is a ligand of selectins involved in inflammatory reactions.[4, 5]

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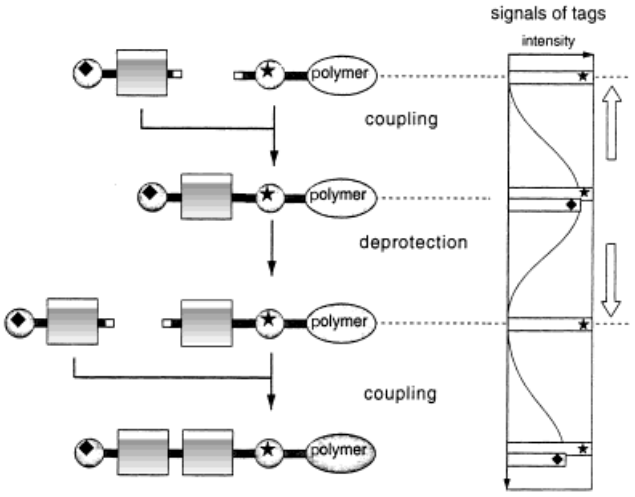
Our strategy is illustrated in Scheme 1. A ^{13}C-enriched linker (∗) is incorporated in the support as a satellite to a ^{13}C-enriched protecting group of the growing molecule (◆). The signal of the protecting group on the growing terminus during



Scheme 1. A strategy designed for quantitatively monitoring the progress of solid-phase oligosaccharide synthesis using gated decoupling ^{13}C NMR spectroscopy with ^{13}C-enriched protecting groups (◆) and an internal ^{13}C standard (∗) in the presence of a relaxation agent.

the coupling reaction can thus be compared to the one from the satellite to determine the completeness of the reaction.

To examine the feasibility of this strategy, we made several decisions prior to the synthesis: 1) At least three glycosylation reactions are carried out on the solid support to give a minimum of tetrasaccharide, which would cover the size of important carbohydrate moieties involved in recognition events. 2) Only two protecting groups—namely, benzyl as "permanent" and ^{13}C-enriched acetyl (◆) as "temporary" protecting groups—are used to avoid any complications. Only the sugar unit added last has other protecting groups. 3) The stereospecificity is controlled by means of solvent effects or by neighboring group participation. 4) ^{13}C-enriched glycine (∗), which is incorporated as a part of the linker, serves as the internal integration standard. 5) All of the donors are given as the thioglycosides.

We started the synthesis with incorporation of [1-^{13}C]glycine **1** into an amine on a Tentagel support (0.26 mmol g^{-1} polymer; **A** →**B**; Scheme 2).[2, 6] The second step, after the Fmoc group had been removed, was to introduce linker **2**, a hydroxyhexanoic acid derivative in which the hydroxyl group was protected with a *1*-^{13}C-labeled acetyl group (◆Ac), to give **D**. The yield was evident from the integration of ^{13}C NMR signals (97.2% yield; see Figure 1). We could have incorporated the first sugar residue as a glycoside of the linker; however, to illustrate the potential of the synthetic strategy and to keep the glycosylation reagents (glycosyl donors) universal, we decided to add the linker first. Removal of the ◆Ac group by treatment of the polymer with MeONa in MeOH/DMF resulted in complete disappearance of the corresponding ^{13}C NMR signal, indicating a quantitative deprotection. Next, the first glycosylation reaction was carried

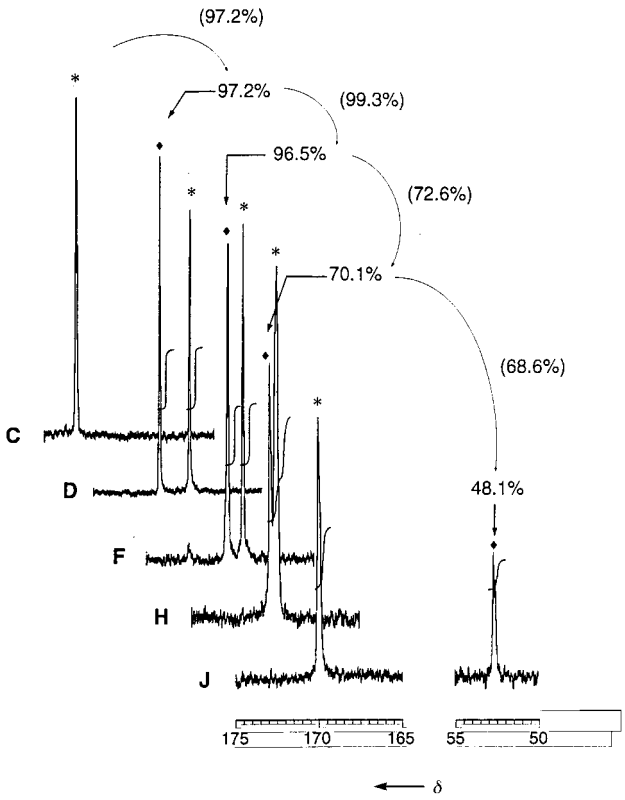


Figure 1. ^{13}C NMR spectra of polymers **C**, **D**, **F**, **H**, and **J**. Only selected areas are shown together with the yields calculated from the integrations. Each reaction yield is shown in brackets. ∗: Signal of the linker (^{13}C-labeled glycine C=O), ◆: signal of the ^{13}C-labeled protecting group in the growing molecule. The height of the signal ∗ in **D** appears lower due to the broadening of the signal.

out with donor **3**,[7] the 4-OH group of which was protected by ◆Ac. The reaction was carried out twice in the presence of DMTST[8] in CH_2Cl_2 at 30 °C to yield **F** (99.3%; see legend of Scheme 2 for abbreviations). To avoid the formation of incomplete sequence, the unreacted hydroxyl group was capped with a TBDMS group. After removal of the ◆Ac group (→**G**), the galactosyl donor **4**[9] was coupled under similar glycosylation conditions and again capped to give the Lewis^x trisaccharide **H** in 72.6%. Deprotection followed by sialylation using donor **5**,[10] the carboxylic acid function of which was protected as a ^{13}C methyl ester, in CH_3CN[11] gave the tetrasaccharide on the solid support (**J**) in 68.4% (overall yield 48.1%).

We have used ^{13}C-enriched carbonyl carbon atoms (◆Ac and ∗glycine) throughout the synthesis except for the terminal sialic acid. This raises a question about the reliability of the integration because the signal of the methyl carbon might be affected by the NOE from the directly attached protons. We finally treated the resin containing **J** with aqueous NaOH to release the tetrasaccharide as well as the ^{13}C-labeled methanol and glycine in a sealed vessel. The released materials were analyzed directly in solution by ^{13}C NMR spectroscopy. The integration of the MeOH signal was found to be 45% (ca. 6% error to gel-phase NMR data shown above) of that of the glycine signal, indicating the usefulness of the gated decoupling method in quantitative solid-phase monitoring. Con-

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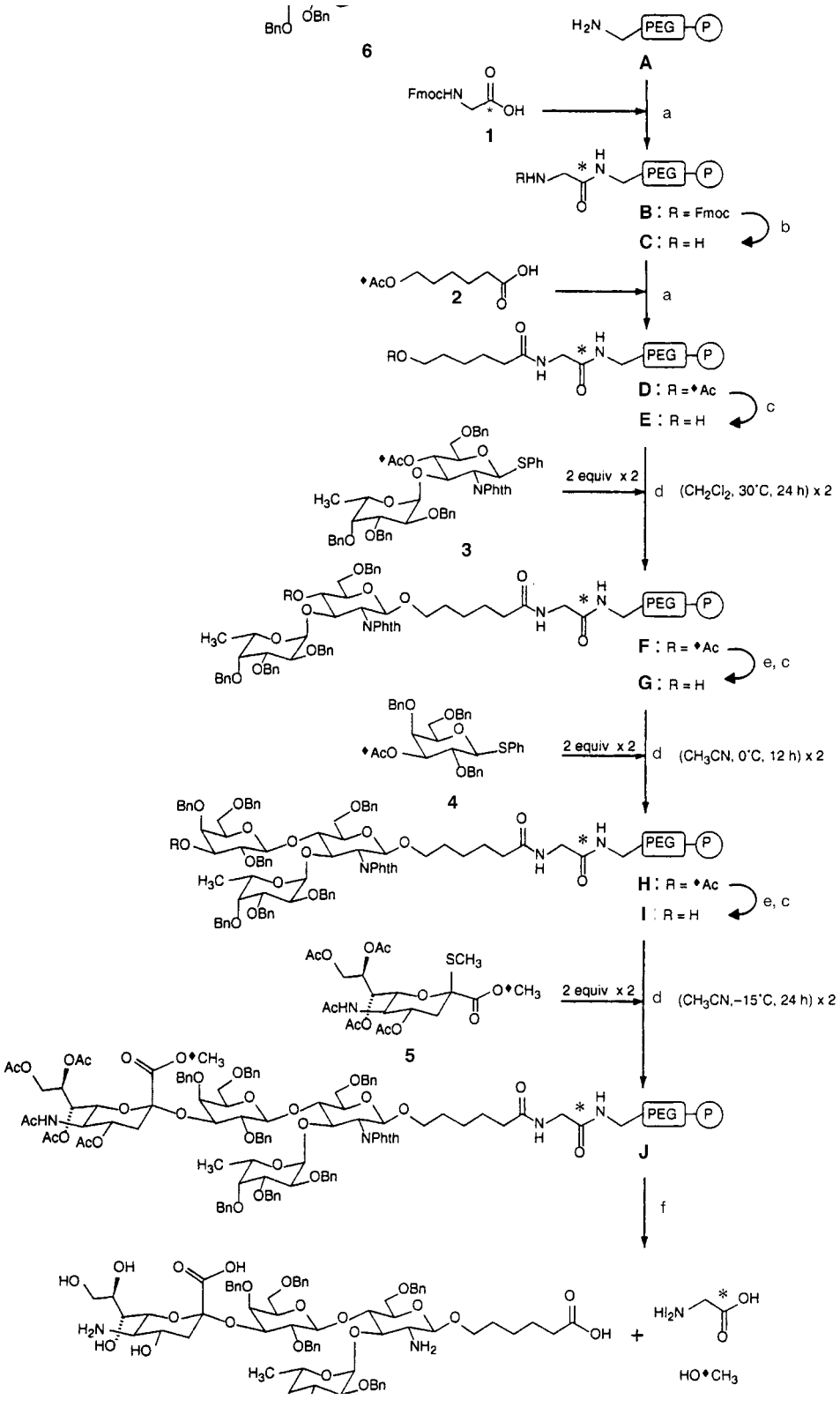

Scheme 2. Solid-phase synthesis of tetrasaccharide **6**. Reagents and conditions: a) HBTU (3 equiv), HOBt (0.2 equiv)/CH_2Cl_2, 25 °C, 3 h: b) 50% piperidine/DMF, 25 C, 3 h; c) 0.05 M NaOMe in MeOH/DMF (1/1 v/v), 25 °C, 24 h; d) DMTST (8 equiv), 3-Å molecular sieves; temperatures, solvents, and reaction times are noted on the scheme; e) TBDMSCl, imidazole, CH_2Cl_2, 25 C, 24 h; f) 1N NaOH in EtOH/H_2O (1/1), 100 °C, 12 h. Bn = benzyl, DMF = dimethylformamide, DMTST = (dimethylthio)methylsulfonium triflate, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = 2-(1-*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole, PEG = poly(ethylene glycol), Ⓟ = Tentagel support, Phth = phthaloyl, TBDMS = *tert*-butyldimethylsilyl.

firmation of the synthesized tetrasaccharide by MALDI TOF mass spectrometric analysis of the released mixture showed the presence of partially protected sialyl Le^x structure **6** (*m*/*z* 1481.8 $[M+H]^+$).

We have used two ^{13}C-enriched markers as an internal standard and as a protecting group on the growing molecule for the simple, nondestructive, and quantitative monitoring of solid-phase chemical oligosaccharide synthesis. Because the two markers are not directly incorporated in the molecule to be synthesized, one may use other tags instead of ^{13}C nuclei for the monitoring. In principle, different ^{13}C-labeled protecting groups can also be used in different orthogonal strategies. Though the anomeric purity of each glycosidic linkage remains to be determined, the strategy should be applicable to the small-scale solid-phase synthesis of oligosaccharides, and perhaps of other molecules, in a combinatorial manner.

Experimental Section

Typical coupling reaction: A suspension of the resin carrying hydroxyl groups (ca. 0.25 mmol g^{-1} resin, 500 mg), phenylthioglycoside (0.25 mmol), and 3 Å molecular sieves (500 mg) in dry CH_2Cl_2 (10 mL) was stirred at 25 °C for 12 h. To this mixture was added DMTST (1 mmol) below the temperature designated in Scheme 2. The reaction mixture was shaken with a vortex mixer at the designated temperature for 24 h. To monitor the course of the reaction, an aliquot of resin was taken from the reaction mixture at this stage. The resin used for the monitoring could be put back to the reaction mixture after it had been washed with $CHCl_3$. After successive washing of the resin with $CHCl_3$, MeOH, water, DMF, and $CHCl_3$, the glycosylation reaction was repeated once more under the same conditions. In order to cap the unreacted hydroxyl group, the resin was treated with *t*$BuMe_2SiCl$ and imidazole at 25 °C for 24 h after washing.

Gated decoupling ^{13}C NMR measurement: The dried resin (ca. 60 mg) was slurried in $CDCl_3$, and the sample was prepared with a relaxation agent,

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chromium(III) 2,4-pentanedionate ([Cr(acac)$_3$], 0.1M] in an ordinary 5-mm ϕ NMR tube. The ^{13}C NMR spectrum was measured on a JEOL EX-270 spectrometer at 68 MHz and operated with 9-s relaxation delay and gated decoupling without NOE (160 transients, 0.6 s acquisition time). The spectra were referred to the resonance for TMS. ^{13}C NMR spin-lattice relaxation times (T_1) were measured by using the inversion recovery method at 298 K (16 data points, 16 scans per point). The T_1 values for methyl and carbonyl groups attached to the resin were shorter than 1 s in the presence of [Cr(acac)$_3$] and 12 s and 29 s in the absence of the relaxation agent.

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[1] a) S. P. Douglas, D. M. Whitfield, J. J. Krepinsky, *J. Am. Chem. Soc.* **1991**, *113*, 5095 – 5097; b) R. Rodebaugh, S. Joshi, B. Fraser-Reid, H. M. Geysen, *J. Org. Chem.* **1997**, *62*, 5660 – 5661; c) P. H. Seeberger, X. Beebe, G. D. Sukenick, S. Pochapsky, S. J. Danishefsky, *Angew. Chem.* **1997**, *109*, 507 – 510; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 491 – 493.

[2] a) E. Giralt, J. Rizo, E. Pedroso, *Tetrahedron* **1984**, *40*, 4141 – 4152; b) A. G. Ludwick, L. W. Jelinski, D. Live, A. Kintanar, J. Dumais, *J. Am. Chem. Soc.* **1986**, *108*, 6493 – 6496; c) F. A. Albericio, M. Pons, E. Pedroso, E. Giralt, *J. Org. Chem.* **1989**, *54*, 360 – 366; d) G. C. Look, C. P. Holmes, J. P. Chinn, M. A. Gallop, *J. Org. Chem.* **1994**, *59*, 7588 – 7590; e) J. Blumel, *J. Am. Chem. Soc.* **1995**, *117*, 2112 – 2113; f) J. M. Brown, J. Ramsden, *Chem. Commun.* **1996**, 2117 – 2118.

[3] J. N. Shoolery, *Prog. Nucl. Magn. Reson. Spectrosc.* **1977**, *11*, 79 – 93.

[4] a) M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori, J. C. Paulson, *Science* **1990**, *250*, 1130 – 1132; b) G. Walz, A. Aruffo, W. Kolanus, M. Berilacqua, B. Seed, *Science* **1990**, *250*, 1332 – 1335; c) J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend, R. M. Marks, *Cell* **1990**, *63*, 475 – 484.

[5] Solid-phase syntheses of sialyl Lewis^x tetrasaccharide were achieved by enzyme reactions: a) M. Schuster, P. Wang, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1994**, *116*, 1135 – 1136; b) O. Blixt, T. Norberg, *J. Org. Chem.* **1998**, *63*, 2705 – 2710.

[6] a) E. Bayer, K. Albert, H. Willisch, W. Rapp, B. Hemmasi, *Macromolecules* **1990**, *23*, 1937 – 1940; b) K. Nokihara, W. Rapp, *Shimadzu Rev.* **1993**, *50*, 25 – 31.

[7] Compound **3** was synthesized through coupling of phenylthioglycosides of tribenzyl L-fucose and *N*-phthaloyl-4,6-benzylidene-D-glucosamine derivative as donor and acceptor, respectively. The synthesis will be reported in due course.

[8] a) P. Fügedi, P. J. Garegg, *Carbohydr. Res.* **1986**, *149*, C9-C12; b) M. Ravenscroft, R. M. G. Roberts, J. G. Tillett, *J. Chem. Soc. Perkin Trans.* **1982**, *2*, 1569 – 1572.

[9] Compound **4** was synthesized according to a literature method: O. Kanie, Y. Ito, T. Ogawa, *Tetrahedron Lett.* **1996**, *37*, 4551 – 4554.

[10] a) O. Kanie, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1988**, *7*, 501 – 506; b) A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, M. Kiso, *Carbohydr. Res.* **1991**, *212*, 277 – 281.

[11] A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1991**, *10*, 493 – 498.

Single-Step Construction of a Nine-Membered Carbocycle by a New [4+4+1] Cycloaddition**

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There is much interest in medium-sized, that is, eight- to eleven-membered, carbocycles because they are often an important structural feature of biologically active compounds. From the synthetic point of view, however, construction of medium-sized ring systems is a formidable objective due to unfavorable entropy factors as well as energy barriers inherent to cyclization (generally ascribed to arising angle strain, bond opposition (Pitzer) strain, and transannular interactions).[1, 2] Especially scarce are methods for the preparation of nine-membered carbocycles,[3] the development of which remains an important challenge in organic chemistry.

Vinylallene has been described to act as a versatile four-carbon unit in metal-catalyzed [4+1][4] and [4+2] cycloaddition[5] reactions. We expected that the use of an appropriate metal catalyst capable of simultaneous multiple coordination of vinylallene would make it possible to incorporate two or more molecules of vinylallene in the resulting skeleton in a single operation. Here we report a palladium-catalyzed [4+4+1] cycloaddition reaction which provides a new route to nine-membered carbocycles. To the best of our knowledge, this is the first example of a [4+4+1] cycloaddition.[6] While our approach presently requires certain substitution in the substrate, we believe that this new reaction may eventually lead to a useful strategy for the construction of nine-membered rings.

A solution of vinylallene **1** in THF was stirred under an atmosphere of carbon monoxide in the presence of 5 mol% palladium(0) catalyst at 30 °C. The reaction mixture was stirred for 43 hours, and vinylallene **1** was consumed to afford a sole product. From the spectroscopic data the structure was concluded to be the symmetrical nine-membered ketone **2**, as depicted in Scheme 1. The cyclic compound **2**, isolated in 87% yield, was assembled from two molecules of vinylallene **1** and one molecule of carbon monoxide. Vinylallene **1** coupled at the 1-position in a head-to-head manner, providing the two four-carbon units. Cheletropic incorporation of carbon monoxide between the 4-positions furnished the nine-membered ketone **2** as a [4+4+1] cycloadduct.

Vinylallene **3** also underwent an analogous [4+4+1] cycloaddition reaction to afford **4** as a solid in 61% yield (Scheme 2). Although the ketone gradually decomposed on exposure to light, careful recrystallization from ether/hexane generated single crystals suitable for X-ray crystallography,[7]

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