[9] G. M. Blackburn, M.-J. Guo, *Tetrahedron Lett.* **1993**, *34*, 149 – 152.
[10] Typical procedure for **8**. A solution of CF_3SiMe_3 (10 mmol) in dry THF (20 mL) was added dropwise at room temperature under nitrogen to a stirred mixture of bis(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite **7** (5 mmol) and cesium fluoride (11 mmol) in dry THF (20 mL). After 4 h the cesium 4-nitrophenoxide salt was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by distillation under reduced pressure.

The Thioglycoside and Glycosyl Phosphite of 5-Azido Sialic Acid: Excellent Donors for the α-Glycosylation of Primary Hydroxy Groups**

Chung-Shan Yu, Kenichi Niikura, Chun-Cheng Lin,* and Chi-Huey Wong*

N-Acetylneuraminic acid (sialic acid, NeuAc) is often found at the nonreducing end of glycoconjugates associated with important biological recognition events.[1] Although many glycosylation reagents are available for the synthesis of sialosides, addition of sialic acid with absolute α-glycosidic linkage remains a significant challenge.[2] Because of the sterically hindered tertiary anomeric center, the presence of an electron-withdrawing carboxyl group, and the lack of a participating auxiliary substituent adjacent to the anomeric center, most of the existing sialyl donors often have relatively low anomeric reactivities, and the sialylation reaction often proceeds with low yield, low α-stereoselectivity and significant undesirable elimination.

In order to tackle these problems, various new sialyl donors, including sialyl phosphites,[3] thioglycosides,[4] and xanthates,[5] have been developed. These donors give high α-selectivity in reactions with secondary hydroxyl groups, but they exhibit low α-selectivity, when primary hydroxyl groups are used as acceptors.[2a] In most cases, the content of undesirable β isomer ranged from 10 to 50% when secondary hydroxyl groups were used as acceptors and was more than 50% with primary hydroxy groups like the 9-OH group of sialic acid as acceptors. Anchimeric assistance by an auxiliary group at C-3[2d, 6] has been demonstrated to improve α-selectivity.

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[**] This research was supported by Academia Sinica (Taipei) and the NIH (USA).

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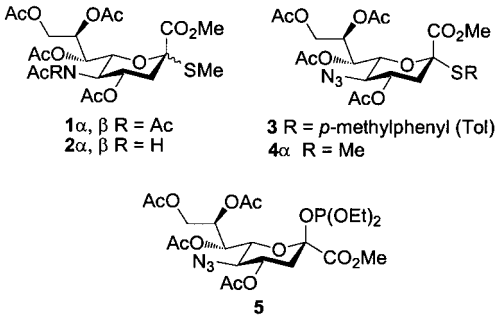
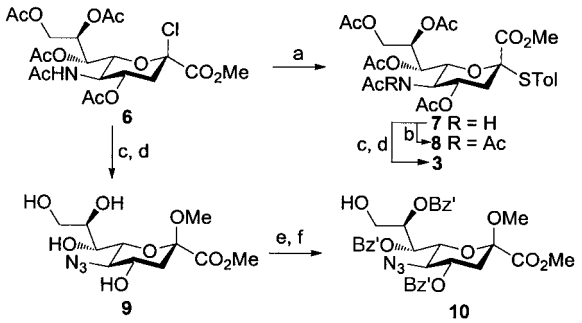
Recently, the di-*N*-acetyl sialyl donor **1** has been shown to exhibit increased reactivity and yield in reaction with the 9- or 8-OH group of an appropriate acceptor,[7] and in certain cases



with higher α-selectivity.[8] The sialic acid 8-OH group was thought to engage in an intramolecular hydrogen bonding interaction with the O atom of the C-1 carbonyl or the 2-OR group, or to interact with the 5-NHAc group of **2**, thus weakening the nucleophilic activity.[7]

This undesirable interaction was circumvented by further acetylation of the NHAc group, and the NAc_2 derivative **1** gave an increased glycosylation yield;[7a] however, the α-selectivity was not improved, and a significant amount of β isomer was present in the product (ca. 30%). We report here the use of the thiosialosides **3** and **4** and the corresponding phosphite **5**, in which the 5-NHAc of **2** is replaced with the azido group, as α-selective glycosylation reagents for primary and secondary hydroxyl groups as acceptors. Sialylation reagents with a 5-azido group have been described in the literature,[9] but their effect on reactivity or stereoselectivity has not been studied, except that a low-yield (ca. 26%) sialylation was reported.[9b]

To prepare the glycosyl donors, the sialyl chloride **6**[10a] was treated with *p*-thiocresol in the presence of Hünig's base to give the α-thioglycoside **7**[10b] in crystalline form (Scheme 1). Subsequent N acetylation of **7** to give **8** could be easily achieved in high yield by treatment with isopropenyl acetate and a catalytic amount of TsOH.[7a] Complete deacetylation[11] of **7** followed by a catalytic diazo transfer[12] and acetylation of the hydroxyl groups gave **3**. The methylsulfanyl-substituted



Scheme 1. a) Thiocresol, $NEtiPr_2$, CH_2Cl_2, RT, 18 h, 85%. b) Isopropenyl acetate, cat. TsOH, 60 °C, 15 h, 95%. c) MsOH, MeOH, 60 °C, 24 h, 55%. d) 1) TfN_3, $CuSO_4 \cdot 5H_2O$, MeOH, RT, 18 h; 2) Ac_2O, py, RT, 4 h (66% for two steps). e) *t*$BuMe_2SiCl$, py, DMAP, CH_2Cl_2, RT, 24 h, then *p*-ClC_6H_4COCl (Bz′Cl), RT, 18 h, 86%. f) HF/py, AcOH, THF, RT, 1 h, 94%.

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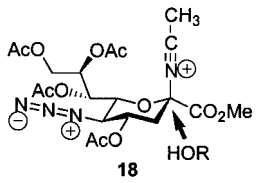
donors **1**[7a] and **2**[13] were synthesized as α/β mixtures according to the reported procedures. The α- and β-5-N_3 methylsulfanyl-substituted donors **4** were obtained from **2** by using similar methods as described in the synthesis of **3**. Under the acidic N,O deacetylation conditions, the Cl substituent of **6** was replaced by OMe; afterwards, the free amine was converted to azide to give **9**, which was further transformed to acceptor **10** as shown in Scheme 1.

We then turned our efforts to investigate the sialylation reactions, which were proceeded under standard reaction conditions. Acceptor **10** (1 equiv) was reacted with donor **3** (2 equiv) in the presence of NIS (3 equiv to donor), TfOH (3–10 mol% to NIS), and molecular sieves (3 Å) in MeCN at $-40\,^{\circ}C$ to give disaccharide **11** with exclusive formation of the hydroxy groups as acceptors (data not shown). The higher α-selectivity may be attributed to the formation of a more stable acetonitrile intermediate, **18**, and the less steric hindrance in the α face caused by the linear azido group.



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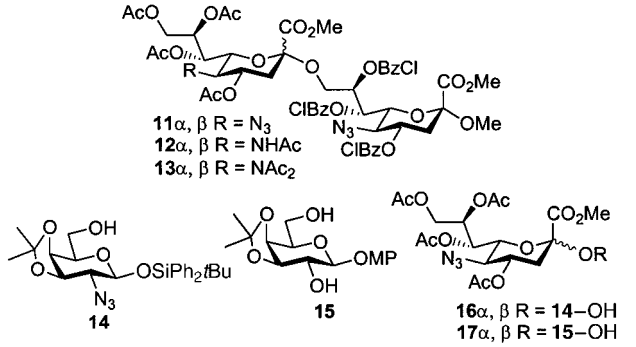
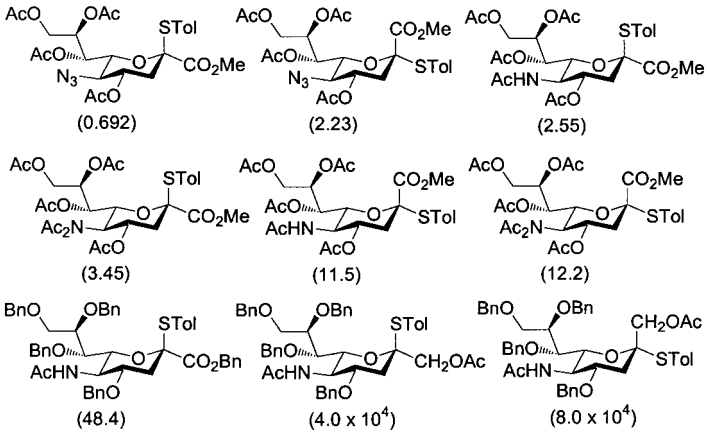
When the reaction solvent was changed to CH_2Cl_2, significant amounts of elimination (from donor) and acyl migration (from acceptor) products were found.

To further understand the azido group effect, we compared the relative reactivity values (measured by HPLC using methanol as acceptor and NIS as activator according to the procedure described previously[14]) of some sialylation reagents (Scheme 2). It is clear that the azido group has a

Table 1. Sialylation reactions leading to products **11**–**17**.



Entry	Donor	Acceptor	Product	Yield [%]	α:β
1	**3**	**10**	**11**α	65	α only
2	**4**α	**10**	**11**α	63	α only
3	**4**β	**10**	**11**α	60	α only
4	**7**	**10**	**12**α,β	75	3:1
5	**2**α,β	**10**	**12**α,β	60	2:1
6	**8**	**10**	**13**α,β	30	4:5
7	**1**α,β	**10**	**13**α,β	40	4:5
8	**3**	**14**	**16**α,β	53	10:1
9	**3**	**15**	**17**α,β	35	3:1



Scheme 2. The relative reactivity values (in parentheses) of sialic acid thioglycosides. The values are based on the corresponding α-thioglycoside of peracetylated mannose.

α isomer in 65% yield (Table 1). To our knowledge, this is the best α-selective sialylation reported so far for the synthesis of the disaccharide Neu5Acα(2→9)Neu5Ac.[2, 4, 7] Both donors **3** and **4** showed similar results (Table 1, entries 1–3), and the anomeric chirality of **4** did not influence α-selectivity and yield (entries 2 and 3). When the same acceptor was reacted with the thioglycoside donors **2** or **7** to give **12**, the α-selectivity decreased (entries 4 and 5). Notably, when acceptor **10** was treated with the di-*N*-acetyl donors **1** or **8** to give **13**, the yield and α-selectivity dramatically decreased (entries 6 and 7).

In order to further demonstrate that the azido donor **3** is a better α-selective donor for primary hydroxyl groups as acceptors, syntheses of the terminal disaccharides sialyl Tn antigen and GM_4 using acceptors **14** and **15** (MP = *p*-methoxyphenyl) were undertaken, and again the α-selectivity was better than with the corresponding 5-NHAc sialyl donors. In addition, the small amount of contaminating 5-azido β isomer can be easily separated by silica gel column chromatography. A similar result was obtained with secondary significant deactivation effect, and both *N*-acetyl and di-*N*-acetyl groups exhibit little difference. Reduction of the anomeric carboxyl group to a hydroxymethyl group remarkably enhances the anomeric reactivity; however, these glycosyl donors gave predominantly the β isomer in glycosidation.[15] Taken together, the azido group effect is apparently both electronic and steric: the linear and electron-withdrawing nature of N_3 stabilizes the reactive axial acetonitrile adduct[2a, 3b, 4] to allow the incoming nucleophile to approach the α face in an SN_2-like reaction.

In order to use the new azido sialyl donors in the synthesis of NeuAcα(2→9)NeuAc containing saccharides, the chemoselective glycosylation strategy[16] was applied. It has been reported that glycosyl phosphites and thioglycosides can be activated by TMSOTf and NIS/TfOH, respectively.[3, 4] The azido phosphite **5** and the azido thiocresol **20** were thus chosen as donor and acceptor, respectively, for the chemoselective glycosylation (Scheme 3). Thioglycoside **3** was treated with NBS followed by diethyl phosphochloridite[17] to give **5**. Compound **20** was obtained from **19** by using a similar method as described in the synthesis of **9**. The glycosylation of **20** by **5** was proceeded in CH_3CN at $-40\,^{\circ}C$ in the presence of TMSOTf to give **21** exclusively in the α

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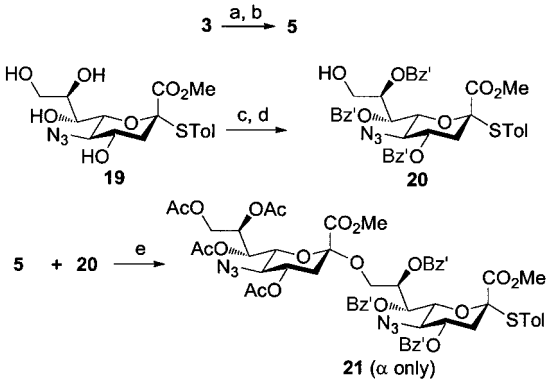


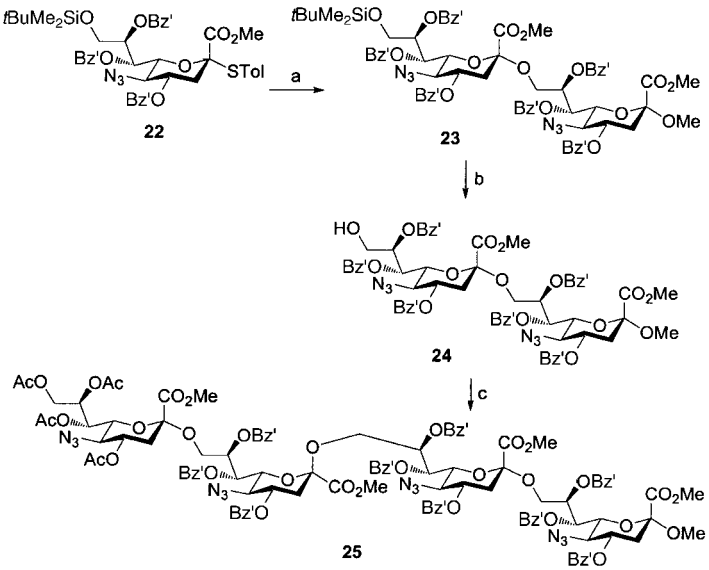
Scheme 3. a) NBS, acetone/H_2O, RT, 1 h, 91%. b) NEt_iPr_2, $ClP(OEt)_2$, CH_3CN, RT, 30 min, 65%. c) tBuMe$_2$SiCl, py, DMAP, CH_2Cl_2, RT, 1 h, then Bz′Cl, 0 °C to RT, 1 h, 88%. d) HF/py, AcOH, THF, 1 h, 89%. e) TMSOTf, 3 Å MS, CH_3CN, −40 °C, 30 min, 51%.

form (51%). Having the thiocresol leaving group, **21** is ready for the next glycosylation. The anomeric configurations of these sialodisaccharides were determined by NMR spectroscopy[7a, 13] based on the chemical shifts of H′-3e (α-glycosides are more downfield than β-glycosides), H′-4 (α-glycosides: $\delta < 5$, β-glycosides: $\delta > 5$), and the value of $\Delta\delta$\{H′9a, H′9b\} (α-glycosides: $\Delta\delta \sim 0.2$ ppm, β-glycosides: $\Delta\delta > 0.3$ ppm) as shown in Table 2.

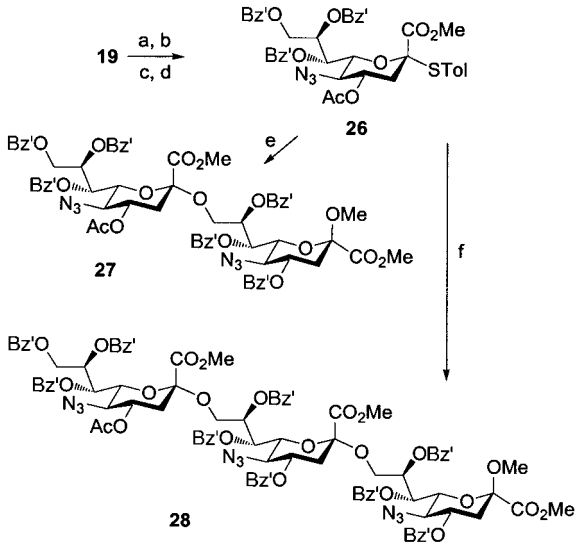
Table 2. Partial ^{1}H NMR assignments of sialyl products.

	$\delta(H'_{3e})$	$\delta(H'_4$	$\lvert\delta(H'_{9a}) - \delta(H'_{9b})\rvert$
11α	2.59	4.72	0.16
21α	2.61	4.74	0.18
12α	2.46	4.75	0.07
23α	2.68	4.94	–
12β	2.31	5.08	0.68
23β	2.49	5.22	–
13α	2.64	5.41	0.20
25	2.57	5.22	–
13β	2.52	5.75	0.55
27α	2.52	4.76	0.39
16α	2.62	4.83	0.10
27β	2.45	5.12	0.51
16β	2.86	5.33	1.54
28α	2.53	4.77	–
17α	2.74	4.86	0.18
28β	2.49	5.14	–
17β	2.56	5.25	0.35
30	2.60	4.72	–

In order to investigate the effect of other protecting groups on the glycosylation stereoselectivity of the 5-azido sialyl donors, compound **22** was prepared from **19** and reacted with acceptor **10** under the standard conditions (Scheme 4). The product **23** obtained, however, contained a significant amount of the β isomer. Surprisingly, coupling of the α-linked disaccharide donor **21** with **24** (obtained from **23** by selective deprotection of the primary hydroxy group) gave only the β product **25**. Using the less hindered donor **26** with OAc at C-4 did not improve the α-selectivity (Scheme 5, for **27** and **28**). Coupling of **3** and **24**, however, gave mainly



Scheme 4. a) **10**, NIS, TfOH, CH_3CN, 3 Å MS, −40 °C, 1 h, 51%, $\alpha:\beta =$ 1.1:1. b) HF/py, AcOH, THF, 1 h, 90%. c) **21**, NIS, TfOH, CH_3CN, 3 Å MS, −40 °C, 1 h, 32%, β.



Scheme 5. a) 2,2-Dimethoxypropane, PTSA, 1 h, 90%. b) Ac_2O, py, −78 °C, 70%. c) 90% AcOH, 50 °C, 1 h. d) Bz′Cl (75%, for c and d). e) **10**, NIS, TfOH, CH_3CN, 3 Å MS, −40 °C, 2 h, 50%, $\alpha:\beta = 5:4$. f) **24**, NIS, TfOH, CH_3CN, 3 Å MS, −40 °C, 40%, $\alpha:\beta = 1:2$.

the α product ($\alpha/\beta = 6/1$) in 60% yield (not shown). In another experiment, compound **21** was converted to the peracetylated donor **29**, which was coupled with **10** to give the trisaccharide **30** in 45% yield with exclusive α-glycosidic linkage (Scheme 6). It appears that bulky substituents could override the azido group effect on the glycosylation stereoselectivity. The optimal sialyl donors for selective α-sialylation of the NeuAc 9-OH group are the 5-azido derivatives with *O*-acetyl protecting groups, including **3**–**5** and **29**.

In conclusion, it has been demonstrated that 5-azido sialyl donors with *O*-acetyl protecting groups are useful α-selective glycosylation reagents, especially for primary hydroxy groups as acceptors. In addition, a chemoselective glycosylation

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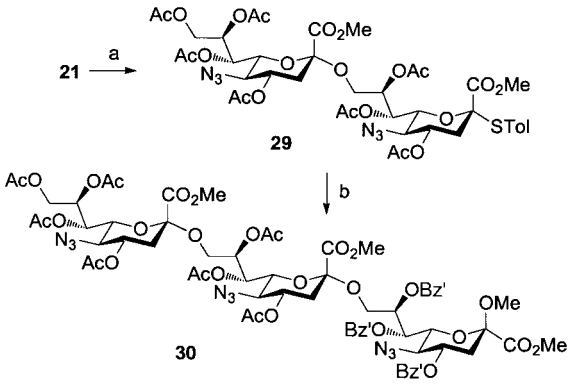

Scheme 6. a) 1) NaOMe, MeOH; 2) Ac_2O, py, RT, 85% for both steps. b) **10**, NIS, TfOH, CH_3CN, 3 Å MS, $-40\,^{\circ}C$, 1 h, 45%, α.

method has been developed for the synthesis of NeuAcα-(2→9)NeuAc as thioglycoside donor for use in subsequent glycosylations. The azido group can be reduced to the NH_2 group for acetylation or incorporation of other substituents. The method described for the synthesis of α-2,9-linked oligomers of sialic acid may find use in the preparation of carbohydrate-based vaccines.[1, 2]

Received: February 23, 2001 [Z16674]

[1] a) *Sialobiology and Other Novel Forms of Glycosylation* (Eds.: Y. Inoue, Y. C. Lee, F. A. Troy II), Gakushin, Osaka, **1999**; b) *Biology of the Sialic Acids* (Ed.: A. Rosenberg), Plenum, New York, **1995**.

[2] a) G.-J. Boons, A. V. Demchenko, *Chem. Rev.* **2000**, *100*, 4539; b) C.-H. Lin, C.-C. Lin in *The Molecular Immunology of Complex Carbohydrates 2* (Ed.: A. M. Wu), Kluwer/Plenum, New York, **2001**; c) M. P. DeNinno, *Synthesis* **1991**, 583; d) K. Okamoto, T. Goto, *Tetrahedron* **1990**, *46*, 5835; e) B. G. Davis, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2137.

[3] a) H. Kondo, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 8748; b) T. J. Martin, R. R. Schmidt, *Tetrahedron Lett.* **1992**, *33*, 6123.

[4] A. Hasegawa in *Modern Methods in Carbohydrate Synthesis* (Eds.: S. H. Khan, R. A. O'Neil), Harwood, The Netherlands, **1996**.

[5] a) K. M. Halkes, P. M. St. Hilaire, A. M. Jansson, C. H. Gotfredsen, M. Meldal, B. G. Davis, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2127; b) A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1990**, *195*, 303; c) V. Martichonok, G. M. Whitesides, *J. Org. Chem.* **1996**, *61*, 1702; d) H. Lönn, K. Stenvall, *Tetrahedron Lett.* **1992**, *33*, 115.

[6] a) J. C. Castro-Palomino, Y. E. Tsvetkov, R. R. Schmidt, *J. Am. Chem. Soc.* **1998**, *120*, 8508; b) V. Martichonok, G. M. Whitesides, *J. Am. Chem. Soc.* **1996**, *118*, 8187; c) T. Ercégovic, G. Magnusson, *J. Org. Chem.* **1995**, *60*, 3378; d) Y. Ito, T. Ogawa, *Tetrahedron Lett.* **1988**, *29*, 3987; e) Y. Ito, S. Nunomura, S. Shibayama, T. Ogawa, *J. Org. Chem.* **1992**, *57*, 1821.

[7] a) A. V. Demchenko, G.-J. Boons, *Chem. Eur. J.* **1999**, *5*, 1278; b) A. V. Demchenko, G.-J. Boons, *Tetrahedron Lett.* **1998**, *39*, 3065.

[8] N. Hossain, G. Magnusson, *Tetrahedron Lett.* **1999**, *40*, 2217.

[9] a) M. A. Sparks, K. W. Williams, C. Lukacs, A. Schrell, C. Priebe, A. Spaltenstein, G. M. Whitesides, *Tetrahedron* **1993**, *49*, 1; b) G. Kuznik, B. Horsch, G. Kretzchmar, C. Unverzagt, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 577.

[10] a) A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1989**, *190*, 317; b) A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1989**, *187*, 35.

[11] T. Sugata, Y. Kan, Y. Nagaregawa, T. Miyamoto, R. J. Higuchi, *J. Carbohydr. Chem.* **1997**, *16*, 917.

[12] P. B. Alper, S.-C. Hung, C.-H. Wong, *Tetrahedron Lett.* **1996**, *37*, 6029.

[13] a) A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, M. Kiso, *Carbohydr. Res.* **1991**, *212*, 277; b) O. Kanie, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1988**, *7*, 501; c) K. Okamoto, T. Kondo, T. Goto, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637; d) H. Paulsen, H. Teitz, *Carbohydr. Res.* **1984**, *125*, 47; e) D. J. M. Van der Vleugel, W. A. R. Van Heeswijk, J. F. G. Vliegenthart-Hart, *Carbohydr. Res.* **1982**, *102*, 121; f) U. Dabrowski, H. Friebolin, R. Brossmer, M. Supp, *Tetrahedron Lett.* **1979**, *48*, 4637.

[14] Z. Zhang, I. R. Ollmann, S.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734.

[15] X.-S. Ye, X. Huang, C.-H. Wong, *Chem. Commun.*, in press.

[16] a) Y. Ito, O. Kanie, T. Ogawa, *Angew. Chem.* **1996**, *108*, 2691; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2510; b) T. Zhu, G.-J. Boons, *Angew. Chem.* **1998**, *110*, 2000; *Angew. Chem. Int. Ed.* **1998**, *37*, 1898.

[17] T. M. Martin, R. Brescello, A. Toepfer, R. R. Schmidt, *Glycoconjugate J.* **1993**, *10*, 16.

Enzyme-Activated Gd^{3+} Magnetic Resonance Imaging Contrast Agents with a Prominent Receptor-Induced Magnetization Enhancement**

Alexander L. Nivorozhkin, Andrew F. Kolodziej, Peter Caravan, Matthew T. Greenfield, Randall B. Lauffer, and Thomas J. McMurry*

Gadolinium-based contrast agents for magnetic resonance imaging (MRI) enhance tissue contrast by increasing the relaxation rate ($1/T_1$) of water protons and are widely used in clinical diagnostics.[1] These compounds are mainly extracellular agents with nonspecific biodistribution. A new generation of contrast agents, currently under development, targets macromolecules associated with specific tissues or disease states, and thereby localizes the agent to the site of interest.[1, 2] Moreover, the binding of the agents to a macromolecule substantially slows molecular rotation of the Gd^{3+} complex resulting in an additional increase in the relaxivity and tissue contrast, a phenomenon known as RIME (receptor-induced magnetization enhancement).[3] The blood pool RIME agent MS-325, currently in Phase III clinical trials for noninvasive angiography, binds noncovalently to human serum albumin (HSA). It greatly reduces extravasation of the agent to surrounding tissue and increases the relaxivity five- to tenfold relative to the relaxivity in the absence of HSA binding.

The scope of targeted MRI agents is potentially limited in that many useful targets associated with disease states are present at nanomolar concentrations, which is too low to be accessible to MRI by the RIME approach alone. One method for localizing a high concentration of an agent at these targets is to exploit an enzymatic activity specific to the tissue or disease state to convert an MRI-silent agent into an activated MRI agent. In a model of this approach, β-galactosidase was used to change the ligand environment around a Gd^{3+} center

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[**] We thank Dr. Shrikumar Nair for helpful discussions.

  1433-7851/01/4015-2903 $ 17.50+.50/0