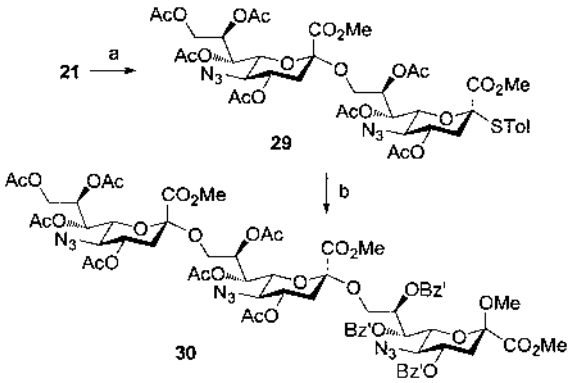

Scheme 6. a) 1) NaOMe, MeOH; 2) Ac_2O, py, RT, 85% for both steps. b) **10**, NIS, TfOH, CH_3CN, 3 Å MS, $-40\,^{\circ}C$, 1 h, 45%, α.

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]

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

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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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and the hydration number of an agent, and resulted in signal enhancement.[4]

We report here a new strategy in which a specific bioactivity can be coupled to the RIME mechanism to generate enhancement of the MRI signal (Scheme 1). The approach relies upon enzymatic transformation of a prodrug Gd^{3+} complex with poor HSA affinity and concomitant low relaxivity (a pro-RIME agent) to a species with improved HSA affinity and enhanced relaxivity. The conversion involved enzymatic removal from the Gd^{3+} complex of a masking group that inhibits HSA binding to expose a binding group with high HSA affinity.

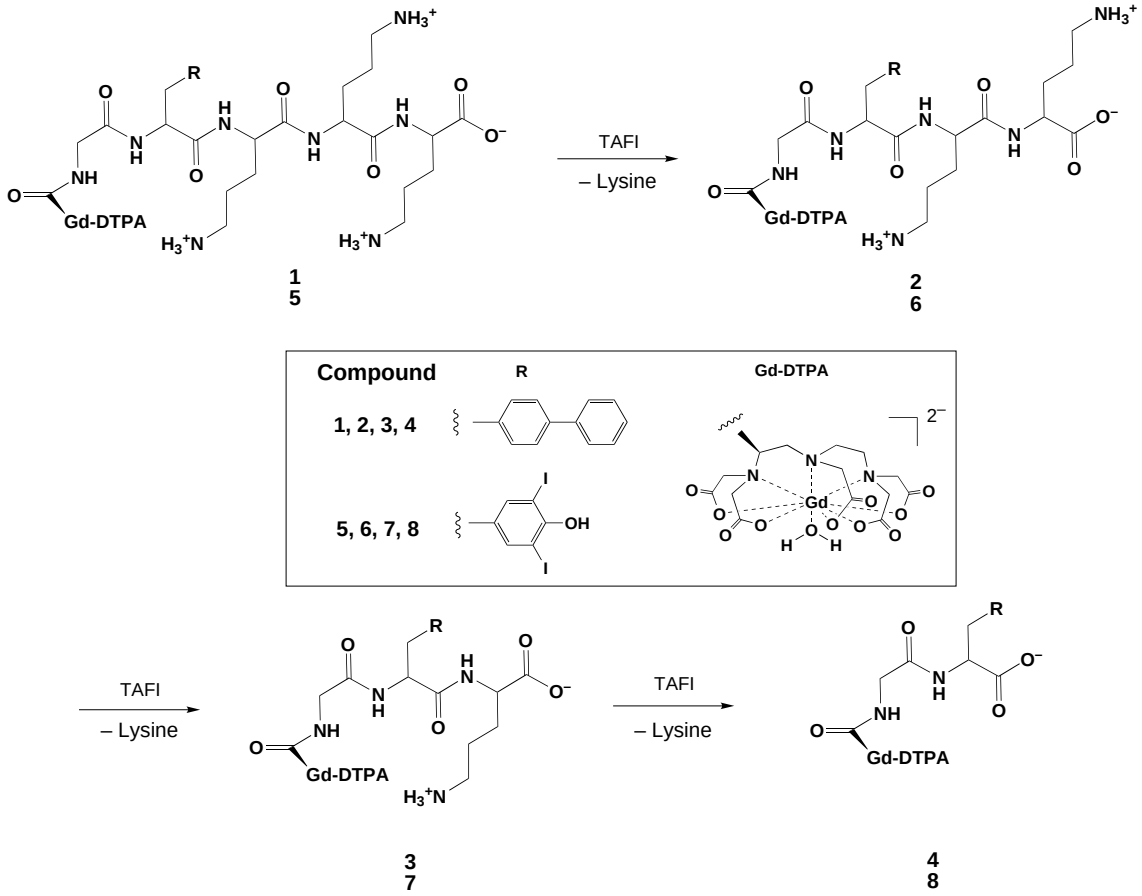
Pro-RIME contrast agents **1** and **5** (Scheme 1) were designed to be cleaved by a human carboxypeptidase B, thrombin-activatable fibrinolysis inhibitor (TAFI). TAFI inhibits clot degradation by cleaving C-terminal lysine residues exposed on fibrin binding sites that are recognized by fibrinolytic proteases such as tPA and plasminogen.[5a,b] This enzyme functions at the crossroads of coagulation and fibrinolysis and has been implicated in thrombotic disease.[5c] The pro-RIME agents are composed of four moieties: 1) a masking group consisting of three lysine residues; 2) an HSA binding group; 3) a glycine linker; and 4) a signal generation group, Gd-DTPA. The trilysine masking group was selected because charged groups have generally poor HSA affinity[6, 7] and the C-terminal lysine residues were expected to be good substrates for cleavage by TAFI. Aryl groups are known to confer high HSA binding affinity,[1, 8, 9] and therefore the diphenylalanine and 3,5-diiodotyrosine residues in complexes **1** and **5**, respectively, were expected to bind well to HSA and enhance the agent's relaxivity when unmasked by TAFI turnover.

The relaxivity and HSA binding data of the trilysine pro-RIME agents at 24 and 37 °C as well as their final products from the enzymatic reaction are given in Table 1. In the absence of HSA, the relaxivities of **1** and the lysine-free product **4** are nearly identical. However, in the presence of 4.5 % (w/v) HSA at 24 °C, the relaxivity of **4** is enhanced by a factor of 30 % over **1** as a consequence of a significantly higher HSA binding affinity; at 37 °C, where the exchange of gadolinium-bound water molecules is more facile, the increase in relaxivity is over 100 %.

Table 1. Relaxivity (20 MHz) and HSA binding data.

Compound	r_1 [$mM^{-1}s^{-1}$] PBS[a]	(24 °C) HSA[b]	r_1 [$mM^{-1}s^{-1}$] PBS[a]	(37 °C) HSA[b]	[%] HSA bound
1	8.4	15.2	7.8	11.1	21.7
4	8.3	19.2	7.7	24.5	69.8
5	9.7	12.5	7.7	9.8	3.9
8	8.1	25.2	7.7	26.5	72.1

[a] Phosphate buffered saline (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4). [b] Human serum albumin (4.5 % w/v).



Scheme 1. Bioactivated Gd^{3+} contrast agents: a Gd^{3+} chelate (signaling domain) is coupled to an HSA binding moiety that is masked by an HSA shielding group. Enzyme activation releases the shielding group and promotes HSA binding.

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Complex **1** is rapidly converted into **4** at physiological concentrations of TAFI (75 nM). The kinetic parameters for the disappearance of **1** ($K_m = 340$ μM, $k_{cat} = 5.3\ s^{-1}$) are comparable to other substrates of TAFI such as hippurylarginine[10] ($K_m = 140$ μM, $k_{cat} = 21\ s^{-1}$). A reaction profile of the turnover of **1** (230 μM) by TAFI (75 nM) in the presence of 4.5% HSA (Figure 1A) was complete within one hour.

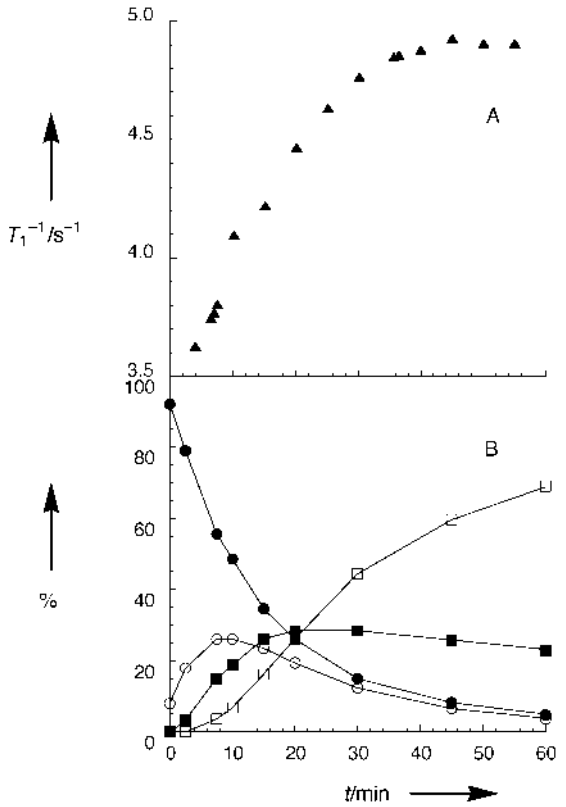

Figure 1. A) Time course of the TAFI-induced change in the relaxation rate of 0.2 mM **1** at 20 MHz in the presence of 4.5% (w/v) HSA. B) Time course of the conversion of **1** into **4**; the distribution of reaction species was quantified by HPLC: **1**: ●, **2**: ○, **3**: ■, **4**: □.

Analysis of the reaction mixture by high-pressure liquid chromatography (HPLC) after quenching at various times confirmed the production of the dilysine and monolysine intermediates **2** and **3**, respectively, as well as **4**. The increases in the $1/T_1$ values most closely paralleled the disappearance of **1** (Figure 1B). The value of $1/T_1$ increased from 3.6 to 4.8 s^{-1} between 0 and 30 min, while 85% of **1** was converted into **4** or intermediates. The value of $1/T_1$ changed only slightly (from 4.8 to 5.0 s^{-1}) between 30 and 60 min, while the concentration of **4** increased by 55% from 100 to 160 μM. Removal of the two C-terminal lysine residues resulted in the majority of the $1/T_1$ increase, whereas removal of the third lysine residue appeared to be less critical for achieving a significant RIME effect.

A second compound, **5**, exhibited a greater TAFI-induced RIME effect than **1**. The relaxivities of **5** and the non-lysine compound **8** in the presence of 4.5% HSA at 24 °C were 12.5 and 25.2 $mM^{-1}s^{-1}$, respectively. Complete conversion of **5** into **8** by TAFI was achieved at a micromolar enzyme concentration and generated an expected 100% relaxivity enhancement as a result of the observed 18-fold increase in the HSA binding activity. A reaction profile of TAFI reaction at nanomolar level (200 μM **5**, 75 nM TAFI, 4.5% w/v HSA), as monitored by the change in $1/T_1$, yielded a smaller effect because of the slower cleavage of the third lysine residue which competed with autoinactivation of TAFI. The monolysine intermediate **7** represented 83% of all the species at 30 min, whereas **8** accounted for 5% of the total. In contrast to **1**, removal of the third lysine residue was essential for attaining the maximal increase in the value of $1/T_1$. At the endpoint of the turnover by TAFI, **7** and **8** represented 44 and 51% of the reaction mixture, respectively, but the $1/T_1$ value had only increased by 26% to 3.8 s^{-1}. Although an approximately threefold relaxivity enhancement can ultimately be reached at 37 °C (Table 1), extended exposures of **5** to TAFI will be required to achieve a beneficial signal enhancement profile in vivo.

In summary, the feasibility of using MRI to detect an enzyme associated with a disease state was illustrated with an efficient pro-RIME contrast agent/TAFI/HSA system that resulted in clinically relevant relaxivity enhancement of the contrast agent. The bioactivation of the MRI agents we have described extends the RIME strategy to the detection of targets present at submicromolar concentrations. Work is in progress to apply this strategy to detecting other protease activities associated with disease states, such as matrix metalloproteinases (MMPs), which have been identified with certain types of cancer, or elastase, an enzyme associated with inflammation sites.

Experimental Section

The DTPA–peptide conjugates **1**, **4**, **5**, and **8** were prepared using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry on a PAC-PEG-PS support with coupling methods based on *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate/*N*,*N*-diisopropylethylamine (HATU/DIPEA). The resin was cleaved in trifluoroacetic acid/triisopropylsilane/water (TFA/TIS/H_2O; 45/1/1) for 2 h and the products purified by reversed-phase HPLC on a C_{18} column using a linear gradient of 0.1% TFA in acetonitrile and 0.1% aqueous TFA. The Gd^{3+} complexes were prepared in aqueous solution by treating the ligands with $GdCl_3$ at pH 5.5–7.5. The final Gd^{3+} concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS). The identity and purity of the ligands and Gd^{3+} complexes was confirmed by electrospray mass spectroscopy (ES-MS) and LC-MS methods.

The progress of enzymatic turnover of **1** and **5** was followed by measuring $1/T_1$ values. TAFI was activated prior to the reaction for 10 min at room temperature as a mixture of TAFI (250 nM) in thrombin (10 nM), thrombomodulin (25 nM) in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, 10 mM), NaCl (150 mM), and $CaCl_2$ (5 mM) at pH 7.5. All enzymes were purchased from Haematologic Technologies, Inc. (Essex Junction, VT). Upon addition of TAFI to the substrate, the reaction contained 75 nM TAFI, 200 μM **1** or **5**, 4.5% HSA, 3 mM HEPES, 7 mM sodium phosphate, 100 mM NaCl, and 1.5 mM $CaCl_2$ in a volume of 1 mL at pH 7.5. Aliquots for HPLC analysis (50 μL) were removed at various time points and quenched by the addition of TFA to a concentration of 1%. Reaction products were identified by HPLC and LC-MS and quantified by peak integration (absorbance at 220 nm). The activity of TAFI rapidly decreases at elevated temperatures[5c] which limited data collection conditions to 2 h at 24 °C.

The percentage of HSA binding was determined by ultrafiltration. The complex (100 μM) was incubated with 4.5% HSA in a solution containing 10 mM sodium phosphate and 100 mM NaCl at pH 7.5 (PBS; 15 min at

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37 °C) and the mixture was filtered in a UltraFree MC 30000 MWCO centrifugal filtration unit (Millipore) at 3500 g for 7 min at 37 °C. The concentration of free substrate in the filtrate was quantified by ICP-MS and the bound fraction was calculated as % bound = ([total]-[free])/[total].

The proton T_1 (longitudinal NMR relaxation time) value of water was measured at 20 MHz at 24 and 37 °C by inversion recovery on a Brucker Minispec; the data were obtained in PBS or with 4.5 % HSA by using 0–40 μM of the Gd^{3+} complex. The relaxivity (r_1) was determined from the slope of the plot of $1/T_1$ versus the sample concentration.

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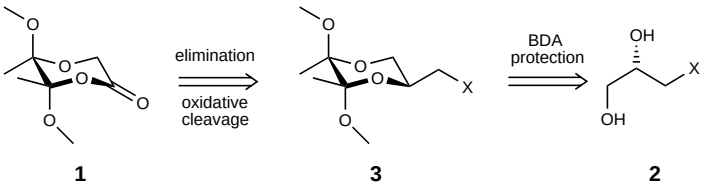
Butane-2,3-Diacetal-Desymmetrized Glycolic Acid—A New Building Block for the Stereoselective Synthesis of Enantiopure α-Hydroxy Acids**

Elena Díez, Darren J. Dixon, and Steven V. Ley*

Of the many classes of functional groups and motifs present in biologically and pharmacologically important compounds, mono- or dialkylated α-hydroxy acids occur commonly.[1–3] As a result of this feature, a range of synthesis methods has appeared over the years.[4–11] A commonly adopted strategy is the α-alkylation of chiral glycolic acid equivalents.[12, 13] Following our earlier reports using dispiroketal desymmetrization for this purpose, we here report the design, preparation, and alkylation reactions of a new chiral glycolic acid equivalent—the butane-2,3-diacetal-desymmetrized glycolate **1**.[14]

Our synthetic plan relied on a chiral memory procedure[15] whereby the chirality of a readily available 3-halopropane-1,2-diol **2** would be used to fix the chirality of the butane diacetal group in the stereoselective protection step.[16] It was envisaged that the alkyl halide product **3** would undergo ready elimination of hydrogen halide to form the *exo*-methylene enol ether,[17] which, after oxidative cleavage, would yield the facially desymmetrized glycolate **1** (Scheme 1).



1 **3** **2**

Scheme 1. Synthetic strategy for the development of a BDA-desymmetrized glycolate equivalent (BDA = butane diacetal).

The initial route employed (*S*)-3-bromopropane-1,2-diol **4** (available by the Jacobsen dynamic hydrolytic resolution of epibromohydrin[18]) as starting material. Treatment with butane-2,3-dione (1.1 equiv) in methanol in the presence of trimethyl orthoformate (2.1 equiv) and camphorsulfonic acid (CSA; 0.1 equiv) at reflux for two hours lead to the BDA-protected alkyl bromide **5** as a single diastereomer in 84 % yield (Scheme 2). To a solution of this material in THF at 0 °C was added an excess (1.2 equiv) of potassium hexamethyldisilazide (KHMDS), which on warming to room temperature overnight, effected a smooth elimination to the desired *exo*-methylene enol ether **6** in 85 % yield. Ozonolysis under standard conditions, followed by triphenyl phosphaine workup, gave the desired building block **1** in 69 % yield as a colorless solid. Recrystallization of this material from diethyl ether/hexanes afforded **1** in > 99 % *ee* as determined by chiral GC.

This route was readily modified to allow synthesis on a multigram scale. Thus, the commercially available and relatively cheap (*S*)-3-chloropropane-1,2-diol[19] **7** was used as the starting material. Standard BDA protection of this compound under the identical conditions described for **5** gave the crude BDA adduct **8** which was treated with an excess of potassium *tert*-butoxide in THF at reflux for 30 minutes. Ozonolysis with a dimethyl sulfide (DMS) workup afforded the crude glycolate product as a colorless solid, which on recrystallization from diethyl ether/hexanes gave enantiomerically pure **1** in 56 % yield over the three steps (Scheme 2).

With multigram quantities of enantiopure **1** available, alkylation reactions were investigated. Initial methylation studies with lithium hexamethyldisilazide (LHMDS) and methyl iodide revealed a strong dependence of the crude

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