adsorbed molecule. Because of the stronger Lewis character of Ti^{IV} and Fe^{III} centers, a larger adsorbate-surface interaction and, consequently, a weakening of the C–O and C-C-O bonds can be expected.[17] Finally, the existence of such an enolate species seems to confirm that the acetone surface condensation reaction occurs through a standard aldol mechanism.

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Chemo-Enzymatic Synthesis of Fluorescent Rab 7 Proteins: Tools to Study Vesicular Trafficking in Cells**

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Proteins that are S-farnesylated and S-geranylgeranylated at C-terminal cysteine residues play critical roles in cell processes such as signal transduction and intracellular trafficking.[1–3] Although our understanding of the biological consequences of protein prenylation has increased significantly over the last few years, there is still relatively little known about the molecular details that govern its functional role, for instance in vesicular trafficking. A particularly relevant example is the role of the Rab proteins in intracellular membrane trafficking. The Rab proteins are a group of small G-proteins that associate with specific membrane components. They are believed to control the events of docking and fusion of intracellular vesicles.[4] Rab proteins are subjected to geranylgeranylation through a process that involves Rab geranylgeranyltransferase (RabGGTase) and an accessory protein termed a Rab escort protein (REP). Thus, in order to undergo prenylation, a newly synthesized Rab protein must bind and form a stable complex with REP, which only then (in contrast to other known prenyltransferases) is recognized by RabGGTase.[5, 6] Upon prenylation the Rab protein remains bound to REP and accompanies it to the corresponding membrane.[7] Subsequent REP-mediated membrane insertion of prenylated Rab proteins is believed to proceed through a putative membrane receptor. The free REP protein is then released and can support another round of Rab prenylation.

Unfortunately, very little is known about the molecular details of this general scenario. Major questions such as whether the lipid groups participate in protein – protein recognition, or the exact mechanism by which the lipidated Rab/REP complex is directed to specific intracellular compartments remain unanswered. Furthermore, despite a number of related reports, the exact affinities of RabGGTase for its lipid substrate are unknown, and the reaction mechanism of the prenylation reaction is also unelucidated. Specific fluorescent probes should enable the dissection of the reaction mechanism through the use of fluorescent spectroscopy. Such probes would allow real-time imaging of the

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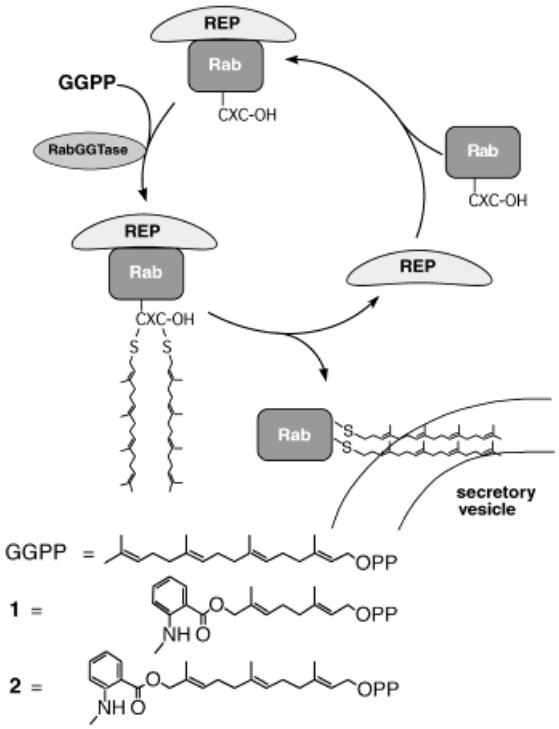
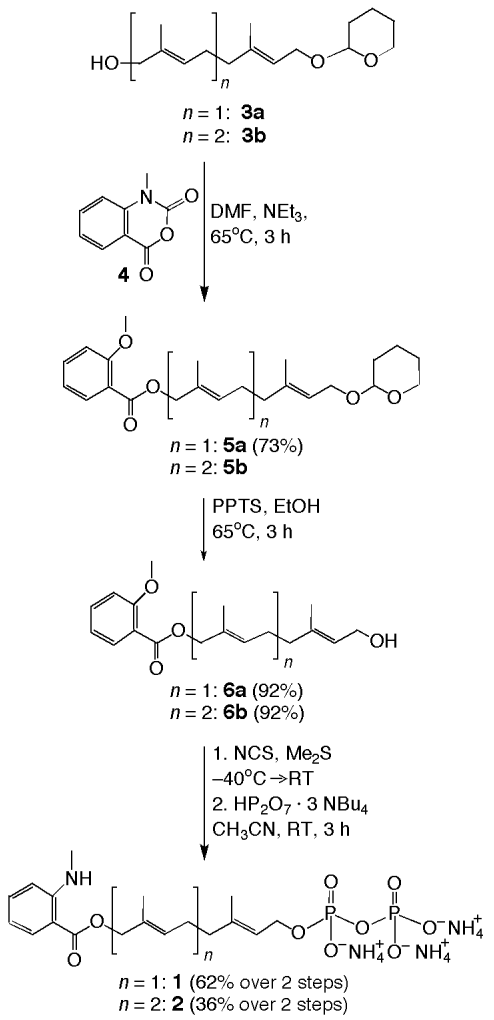
reaction processes and thus be superior to radioactive prenyl derivatives. These compounds are expected to be especially relevant for the study of the transient kinetics of the isoprenoid:prenyl transferase interaction or prenylation reaction. It is also very appealing to use fluorescent isoprenoids or fluorescentely prenylated proteins for the study of the prenylation reaction and function of the prenylated proteins in vivo.[8]

Here we describe the synthesis of two *N*-methylanthraniloylisoprenoid diphosphate derivatives, compounds **1** and **2** (Scheme 1). These compounds bind to RabGGTase and are



Scheme 1. Intracellular processing of the Rab proteins, through the use of geranylgeranylation of the C-terminal cysteine residues. A comparision of the size of GGPP and the fluorescent derivatives **1** and **2** is also shown.

transferred efficiently to Rab 7. The fluorescent Rab 7 proteins thus generated may provide important tools for further biological studies on vesicular trafficking in cells. The fluorescent *N*-methylanthranilate group was chosen at the onset of the synthesis as it is one of the smallest known compounds that produces relatively intense fluorescence upon irradiation with UV light. The synthesis of the novel, fluorescently labeled analogues starts from the known alcohols **3a** and **3b**,[9, 10] both of which are obtained in a two-step sequence from commercially available geraniol and farnesol (Scheme 2). Attachment of the fluorescent *N*-methylanthranilate group[11] was achieved in good yields by acylation of the allylic alcohols with *N*-methylisatoic anhydride (**4**). Removal of the tetrahydropyran protecting group under acidic conditions furnished the desired fluorescent alcohols **6a** and **6b** in excellent yields. Finally, conversion of these compounds into the target diphosphates was achieved by chlorination[12] (which also proceeded in excellent yields), followed by displacement of the allylic chlorides with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate.[13] The diphosphates **1** and **2** were isolated after ion exchange chromatography on Dowex, and then purified by flash chromatography (RP18 silica gel with gradient elution). Proof of the diphosphates structure was obtained by ^{1}H NMR, ^{31}P NMR, and ^{13}C NMR spectroscopy, as well as by FAB mass spectrometry.[14–16]



Scheme 2. Synthesis of **1** and **2**. NCS = *N*-chlorosuccinimide, PPTS = pyridinium-*p*-toluenesulfonate, RT = room temperature.

Analysis of the excitation/emission spectra of both compounds revealed that, as expected from the type of reporter group used, the fluorescence had the excitation maximum at 340 nm and an emission at 450 nm. Subsequently performed kinetic experiments showed that both diphosphates **1** and **2** bind to RabGGTase, with **2** binding better than **1**.[17] The affinity difference between both compounds was consistent with the size difference, as compound **2** has essentially the same length as geranylgeranyl diphosphate (GGPP), while **1** is closer in length to farnesyl diphosphate (FPP; Scheme 1). To determine if both derivatives could substitute for GGPP in the enzymatic protein prenylation we investigated if the fluorescent derivatives could be transferred enzymatically to a Rab protein by RabGGTase (Scheme 3).[18] Rab 7, a protein that controls the biogenesis of late endosomes, was chosen as a substrate.[19] To rule out the possibility of unspecific

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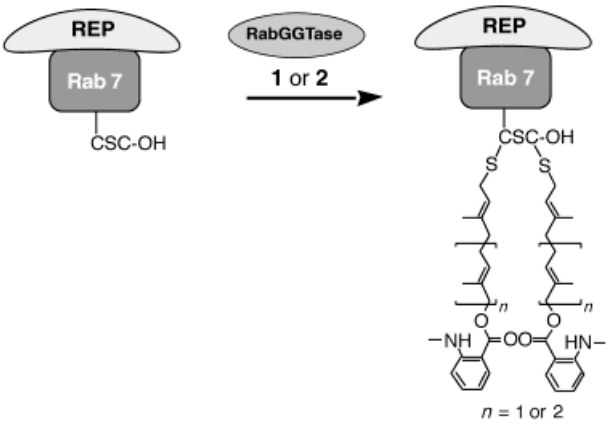

Scheme 3. Enzymatic transfer of **1** and **2** onto Rab 7 by RabGGTase and REP-1.

incorporation of the analogues we performed control reactions without REP-1, as it had previously been demonstrated that REP-1 is essential for catalytic activity of RabGGTase.[6, 20] As an additional control the reactions were supplemented with GGPP as a competitive substrate. After an incubation period the proteins were precipitated with trichloroacetic acid, washed with acetone to remove the excess of free lipid, and finally separated on a SDS-PAGE gel. Fluorescently labeled proteins could be visualized by excitation of the *N*-methylanthraniloyl group at 340 nm by a fluorescence scanner. As shown on Figure 1 there was an easily detectable

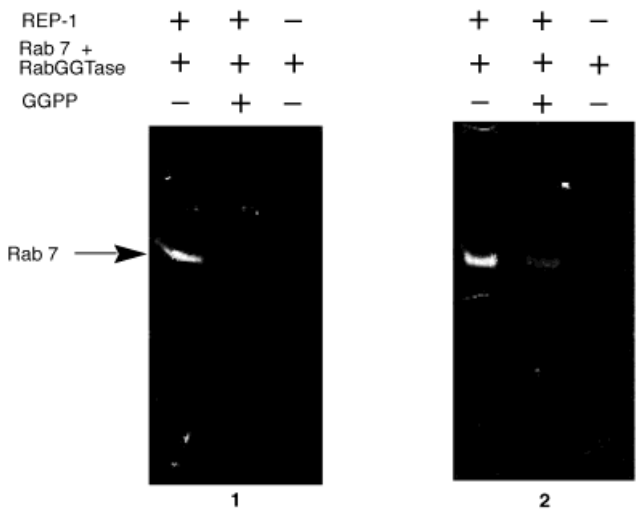

Figure 1. Results of the SDS-PAGE gel of the fluorescently labeled Rab 7 protein after prenylation with the fluorescent lipid derivatives **1** and **2**, plus control reactions.

fluorescent band of about 26 kDa. Coomassie staining of the gel confirmed that fluorescence was localized with the Rab 7 band (data not shown). There was no detectable fluorescence in the samples that lacked REP-1. Finally, fluorescence of the control reactions supplemented with GGPP was suppressed, either by more than 70% in the case of **2**, or to an undetectable level in the case of **1**. The observed inhibition is consistent with the difference in binding affinities of RabGGTase for compounds **1** and **2**. In the absence of the competing GGPP there was, however, no observable difference in prenylation efficiency with both analogues. This suggests that both compounds were transferred efficiently at the chosen concentrations.

In conclusion, we have synthesized two novel fluorescent derivatives of geranylgeranyl diphosphate. These compounds could be efficiently attached to the vesicular trafficking protein Rab 7 through an enzymatic transfer reaction that requires both RabGGTase and REP-1. It is believed that **1** and **2** should pave the way to the elucidation of the functional mechanism of RabGGTase. Moreover, the fluorescent Rab 7 proteins can now be utilized for in-vivo studies on vesicular trafficking processes. However, the interaction of fluorescent Rab 7 with lipid bilayers remains to be tested to determine if the introduction of a hydrophilic ester has any effect on the overall binding affinity.

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[14] Diphosphate **1** was obtained as a pure white powder, and was stored at −78 °C until required: Characteristic data: $R_f = 0.8$ (*i*PrOH/25 mM NH_4HCO_3 1:3 on RP18 silica gel); UV (H_2O): λ_{max} (ε) 220 (27076), 251 (7971), 355 nm (4817); ^{1}H NMR (500 MHz, D_2O): $\delta = 7.67$ (m, 1H), 7.22 (m, 1H), 6.44–6.57 (m, 2H), 5.26–5.35 (m, 2H), 4.45 (s, 1H), 4.41 (s, 1H), 4.29 (t, $J = 6.3$ Hz, 2H), 2.67, 2.63 (2s, 3H), 1.87–2.04 (m, 4H), 1.53, 1.49 (2s, 3H), 1.52 (s, 3H), NH signal was not observed; HR-MS (FAB) calcd for $C_{18}H_{28}NO_9P_2$ $[M+1]^+$: 464.1239; found: 464.1294.
[15] Diphosphate **2** was obtained as a white powder, and was stored at −78 °C until required: Characteristic data: $R_f = 0.5$ (*i*PrOH/25 mM NH_4HCO_3 1:3, on RP18 silica gel); UV (H_2O): λ_{max} (ε) 194 (32498), 220 (23235), 253 (7340), 353 nm (5236); ^{1}H NMR (500 MHz, D_2O): $\delta = 7.58$ (br d, $J = 7.7$ Hz, 1H), 6.96 (m, 1H), 6.19–6.26 (m, 1H), 5.14–5.24 (m, 2H), 4.78 (br s, 1H), 4.25–4.36 (m, 4H), 2.45 (m, 3H), 1.67–1.80 (m, 8H), 1.45 (s, 3H), 1.34 (s, 3H), 1.25 (s, 3H), NH signal was not observed; HR-MS (FAB) calcd for $C_{23}H_{36}NO_9P_2$ $[M+1]^+$: 532.1865; found: 532.1930.

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[16] It is interesting to note that a doubling of the signals in both the ^{1}H and ^{13}C spectra was observed in the NMR spectra of the diphosphate compounds. This result was found to be concentration and substrate dependent, and is thought to reflect slow rotation about the vinylogous carbamate bonds. The doubling of signals was especially prevalent in the farnesyl derivative **2**, and was observed, although to a less degree (ca. 10–15%), in the intermediates that lead up to the farnesyl diphosphate derivative (**5b** and **6b**). The doubling of the NMR signals however, was not observed for the geranyl intermediates (**5a** and **6a**).

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[18] Standard prenylation assay: 50 µl of the assay contained: 120 pmol Rab 7, 120 pmol REP-1, 120 pmol RabGGTase, and 2.4 nmol of either **1** or **2**. Buffer: 20 mM Hepes pH 7.2, 20 mM NaCl, 5 mM 2-sulfanylethanol (BME), 1 mM NP-40. The mixture was incubated at 37 °C for 20 minutes and the reaction stopped by diluting the samples with cold H_2O and precipitating the protein with 10% trichloroacetic acid. The protein pellet was washed with cold acetone and re-suspended in SDS-PAGE loading buffer. Proteins were resolved on the 15% SDS-PAGE gel and the prenylated proteins were visualized with a Fluor 100 (BioRad) fluorescence scanner.

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Tetravalent Tellurium Ligands

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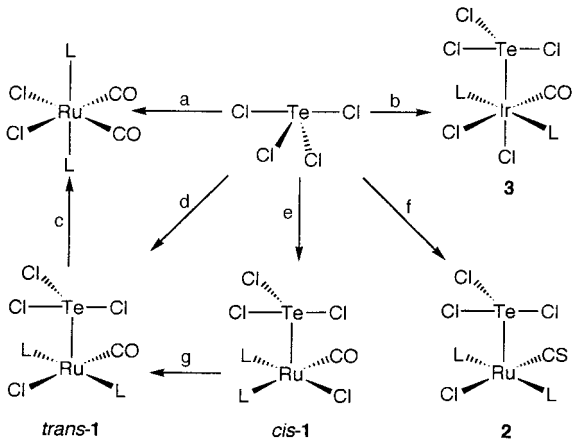
The comparatively sparse chemistry of tellurium-donor ligands typically involves the chalcogen being formally in the divalent state, that is, telluroethers and tellurolates. In recent times, a substantial group of compounds involving "naked" (i.e., substituent-free) tellurium has emerged,[1] heralded by the unsurpassed elegance of the complex $[Te\{Mn(CO)_2(\eta\text{-}C_5H_5)\}_3]$ discovered by Herberhold et. al.[2] Clearly, oxidation states are however of limited use in describing such compounds. Well-defined ligands based on dative tellurium in higher oxidation states are however unknown, although Whitmire and Eveland have recently reported the Zintl cluster $[Fe_2(CO)_6(\eta^2{:}\mu_2,\mu_2\text{-}Te_4)(\mu\text{-}TeCl_2)]$, wherein the "$TeCl_2$" bridge might be described as based on either di- or tetravalent tellurium.[3] Tetravalent sulfur ligands are of course well known in the form of sulfoxides, sulfur dioxide, sulfines, sulfur diimides, and iminooxosulfuranes,[4] however such compounds based on tellurium are either transient or oligomeric in nature. Complexes of the SF_3 and Se(=O)Cl ligands have been previously reported from the oxidative addition of SF_4 or $O{=}SeCl_2$ to $[IrCl(CO)(PEt_3)_2]$[5] or $[IrCl(CO)(PPh_3)_2]$,[6] respectively. A Lewis base adduct of $TeCl_4$ with the pentacarbonyl manganate anion has also been described very recently.[5b] Herein we report the first mononuclear transition metal complexes ligated by the tetravalent trichlorotelluronium group. These result from the reactions of $[IrCl(CO)(PPh_3)_2]$ or $[Ru(CH{=}CH_2)Cl(CA)(PPh_3)_2]$ (A = O, S) with tellurium tetrachloride.

We encountered the first trichlorotelluronium ligand in the product of the reaction of $[Ru(CH{=}CH_2)Cl(CO)(PPh_3)_2]$[7] with $TeCl_4$. In addition to polyvinylchloride, a bright yellow complex formulated as $[Ru(TeCl_3)Cl(CO)(PPh_3)_2]$ (**1**) is obtained in 37% yield after recrystallization (Scheme 1). If the preparation is carried out at room temperature a 1:1 mixture of *cis*/*trans*-bis(phosphane) isomers results; however, at 50 °C the *trans*-bis(phosphane) arrangement is formed exclusively. The same reaction with $[Ru(CH{=}CH_2)Cl(CS)(PPh_3)_2]$ however provides only the isomer of $[RuCl(TeCl_3)(CS)(PPh_3)_2]$ (**2**) with *trans*-coordinated phosphanes. It is noteworthy that whilst the $TeCl_3$ ligand in **1** rotates freely at room temperature (singlet ^{31}P resonance), an apparently static structure is adopted for **2** ($^{31}P_A{}^{31}P_B$ system, *trans*-$^{2}J(AB) = 335$ Hz). This is consistent with the enhancement of a (presumably weak) π-dative component to the Te–Ru interaction to the more π-acidic but isosteric ruthenium center in **2**. The subsequent chemistry of complexes **1** and **2** has so far proven disappointing in that all attempts to introduce phosphanes, isocyanides, or even CO(!) as a sixth ligand (and thereby coordinative saturation) at ruthenium resulted in deposition of elemental tellurium. Similar deposition of tellurium occurs on treatment with amines or alcohols.



Scheme 1. Reagents and conditions (25 °C unless otherwise indicated, L = PPh_3): a) $[Ru(CO)_2L_3]$, C_6H_6; b) $[IrCl(CO)L_2]$, THF; c) CO, CH_2Cl_2; d) $[Ru(CH{=}CH_2)Cl(CO)(PPh_3)_2]$, C_6H_6, 50 °C; e) $[Ru(CH{=}CH_2)Cl(CO)(PPh_3)_2]$, C_6H_6; f) $[Ru(CH{=}CH_2)Cl(CS)(PPh_3)_2]$, C_6H_6; g) $CDCl_3$, seven days.

This apparent lack of synthetic utility, coupled with our failure to obtain crystallographic grade crystals of **1** or **2**, led us to explore alternative examples of this ligand. As noted above, the complexes $[IrCl(CO)(PR_3)_2]$ (R = Et, Ph) oxida-

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