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Asymmetric Synthesis of RK-682 and Its Analogs, and Evaluation of Their Protein Phosphatase Inhibitory Activities.

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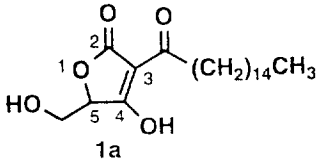
Abstract: We report an asymmetric synthesis of a potent tyrosine phosphatase inhibitor, RK-682 and its analogs. The absolute stereochemistry of RK-682 was determined to be (*R*). The inhibitory activities of RK-682 and its analogs, (*R*)-**1a**, (*S*)-**1a**, (*R*)-**1b** and (*R*)-**1c** toward various protein phosphatases (VHR, cdc25A, cdc25B, and PP1) are also reported.

RK-682 was recently isolated from *Streptomyces* sp. 88-682 as a tyrosine phosphatase inhibitor and was found to have quite interesting biological activities.[1] RK-682 arrests cell cycle progression at the G_1 phase of mammalian cells[1b] and enhances ATP-induced long-term potentiation in CA1 neurons of guinea-pig hippocampal slices.[1c,d] The inhibitory effects of RK-682 on protein phosphatases (VHR and CD45) suggest that protein phosphorylation might be involved in these important biological processes. RK-682 could be a powerful biological tool for studying these important signal transduction processes and may also be a lead compound for the development of therapeutic agents such as anti-tumor drugs. The chemical structure of RK-682 was assigned as 3-hexadecanoyl-5-hydroxymethyltetronic acid (**1a**). The same structure was assigned by the Takeda group to a compound isolated from cultures of *Streptomyces* sp. AL-462 that inhibits the activity of phospholipase A_2.[2] They also reported a synthesis of (+)-**1a** from D-ribose to confirm that their compound has the (*R*)-configuration. The optical rotation of Takeda's material was reported to be +58.5°, whereas that of RK-682 was -90.1° causing confusion about the structure and absolute stereochemistry of these compounds. The CHIBA-GEIGY group has reported isolation of the sodium salt of **1a** from *Actinomycete* strain DSM7357 and its inhibition of HIV-1 protease, but they did not report its optical rotation and absolute stereochemistry.[3] With this background, we planned to develop an efficient and flexible synthetic route to RK-682 to clarify this structural problem and to synthesize a variety of analogs. Here we report the synthesis and determination of absolute stereochemistry of RK-682. The synthesis and inhibitory activity of several analogs toward protein phosphatases are also reported.

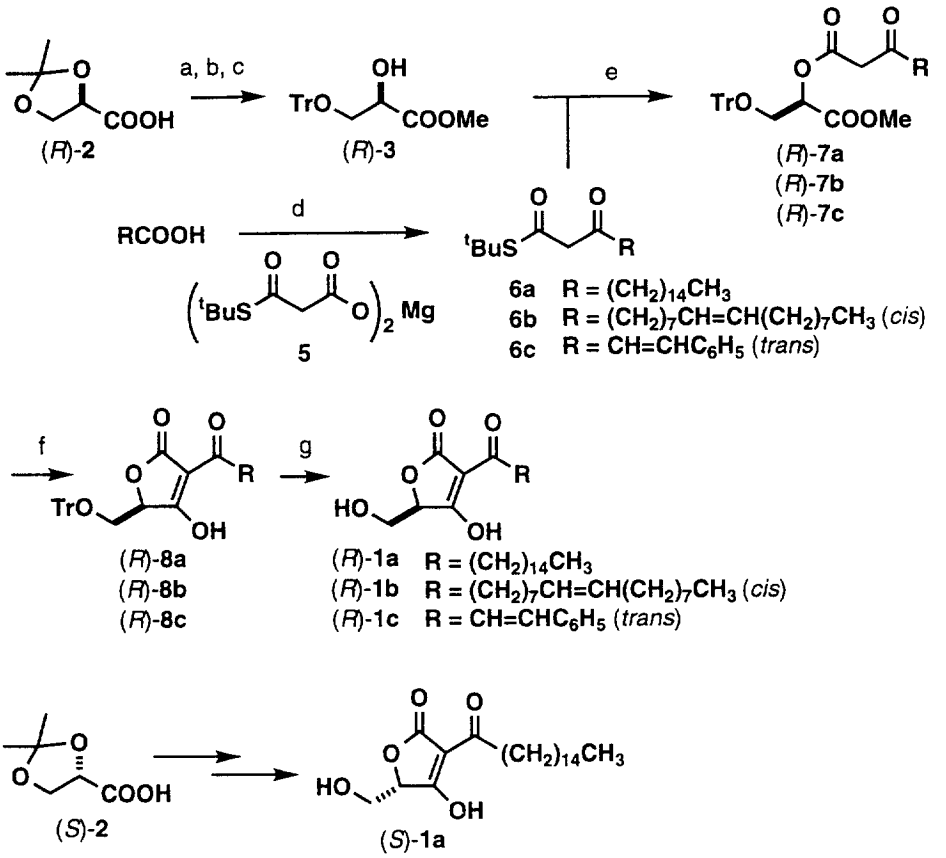


1a

Figure 1

The optically active glyceric acid derivatives (*R*)-**2** and (*S*)-**2**, readily prepared from inexpensive D-mannitol and L-ascorbic acid respectively, were chosen as starting materials.[4] Construction of the key 3-acyltetronic acid skeleton was achieved by using the procedure reported by Ley *et al.* as shown in Scheme 1.[5] Specifically, (*R*)-**2** was first converted to the hydroxyester (*R*)-**3** via methylation with diazomethane,

deprotection of the acetonide group, and selective protection of the primary alcohol with a trityl group. The β-ketoester group was introduced by the silver salt promoted condensation of (*R*)-**3** with thioester **6a** to give (*R*)-**7a** in 74% yield. The thioester **6a** was synthesized from hexadecanoic acid using the magnesium salt of the malonic acid derivative **5**.[6] Cyclization of the β-ketoester (*R*)-**7a** proceeded smoothly with tetrabutylammonium fluoride to give the desired 3-acyltetronic acid (*R*)-**8a** ($[\alpha]_D^{20}$ +48.3° (*c* 1.02, $CHCl_3$)) in 95% yield. Deprotection of the trityl group afforded (*R*)-**1a** in 75% yield. All spectral data including the optical rotation ($[\alpha]_D^{20}$ +58.1° (*c* 0.47, $CHCl_3$)) and melting point (105-108 °C) of synthetic (*R*)-**1a** were identical with that reported by the Takeda group[2] but inconsistent with that reported for RK-682.[1a,7]



(a) CH_2N_2, Et_2O, 0 °C, 82%; (b) 1N aq. HCl, MeOH, 23 °C; (c) Ph_3CCl, NEt_3, cat. DMAP, 23 °C, 60% (2 steps); (d) CDI, THF, 23 °C, then **5**, 93% (**6a**), 100% (**6b**), 53% (**6c**); (e) CF_3COOAg, **6**, THF, 23 °C, 74% (**7a**), 53% (**7b**), 68% (**7c**); (f) Bu_4NF, THF, 23 °C, then 6N aq. HCl, 95% (**8a**), 92% (**8b**), 53% (**8c**); (g) 1N aq. HCl, MeOH, 75% (**1a**), 69% (**1b**), 87% (**1c**).

Scheme 1

In the course of these studies we realized that upon treatment of (*R*)-**1a** with silica gel (MeOH-$CHCl_3$), an unknown, less soluble compound was obtained. The ^{1}H-NMR spectrum of this compound was found to be identical to that of natural RK-682. The $[\alpha]_D$ value of this compoud was quite unsteady, and in

some cases, the opposite sign of the rotation was observed. Since (*R*)-**1a** was recovered by the treatment of this compound with 0.5 N aqueous hydrochloric acid, it might be a silica complex of (*R*)-**1a**.[8] Furthermore, we have found that treatment of the natural RK-682 with 0.5 N aqueous hydrochloric acid afforded a compound whose spectral data and optical rotation ($[\alpha]_D^{20}$ +58.1° (*c* 0.16, $CHCl_3$)) were identical to the synthetic (*R*)-**1a**. Since silica gel column chromatography (MeOH-$CHCl_3$) was employed in the purification of RK-682, it is likely that RK-682 was isolated as a silica complex of (*R*)-**1a**. These observations likely explain the difference in optical rotation between natural RK-682 and (*R*)-**1a**.

Using the same synthetic route, (*S*)-**1a** ($[\alpha]_D^{20}$ -58.4° (*c* 0.50, $CHCl_3$), mp 105-108 °C) was synthesized from (*S*)-**2**. The novel side chain analogs (*R*)-**1b** and (*R*)-**1c** were also synthesized as shown in Scheme 1.

We next investigated the ability of **1a–1c** to inhibit dual-specificity protein phosphatases. As shown in the Table 1, (*R*)-**1a**, (*S*)-**1a**, and natural RK-682 have similar activity against VHR, a dual-specificity protein phosphatase which was isolated from a human fibrobrast,[9,1b] suggesting that the stereochemistry at the C_5-position is not important. The analog (*R*)-**1b** with an unsaturated side chain also showed similar activity. On the other hand, the cinnamoyl derivative (*R*)-**1c** was inactive. This fact indicates that the hydrophobic side chain at the C_3-position plays an important role in the recognition by this enzyme. We also examined the ability of these compounds to inhibit cdc25A and cdc25B,[10] dual specificity protein phosphatases that play important roles in cell cycle regulation by dephosphorylating cdc2-cyclin complexes. Although natural RK-682 and (*R*)-**1c** did not inhibit these enzymes, the synthetic free tetronic acid derivatives (*R*)-**1a** and (*S*)-**1a** did slightly (IC_{50} 100 μM). More interestingly, (*R*)-**1b** inhibited the phosphatase activity of both cdc25A and cdc25B with an IC_{50} of 34 μM. These facts suggest that the enzyme specificity can be changed by modification of the C_3-side chain. None of these compounds inhibited protein serine/threonine phosphatase1 (PP1).[11] These highly selective inhibitors **1a** and **1b** could be valuable biological tools to study the signal transduction pathways relevant to cell cycle progression.

Table 1. Inhibitory Activity of 3-Acyltetronic Acid Derivatives to Protein Phosphatases.[9–11]

	IC_{50} (μM)			
Compound	VHR	cdc25A	cdc25B	PP1
RK-682 (natural)	1.0	>100	>100	>100
(*R*)-**1a**	1.5	100	100	>100
(*S*)-**1a**	1.4	100	100	>100
(*R*)-**1b**	3.4	34	34	>100
(*R*)-**1c**	>100	>100	>100	>100

In summary we have synthesized (*R*)-**1a**, its enantiomer (*S*)-**1a**, and its side chain analogs (*R*)-**1b** and (*R*)-**1c**. We have also found that the treatment of synthetic (*R*)-**1a** with silica gel (MeOH-$CHCl_3$) gave a silica complex of (*R*)-**1a**, which is identical to the natural RK-682. The absolute stereochemistry at the C_5-position of RK-682 was determined to be (*R*) by the comparison of the optical rotation of synthetic (*R*)-**1**

with the acid-treated natural RK-682. Evaluation of ability of synthetic (*R*)-**1a**, (*S*)-**1a**, and its analogs, (*R*)-**1b** and (*R*)-**1c** to inhibit protein phosphatases has indicated that the stereochemistry at the C_5-position is not important, but the hydrophobic side chain at the C_3-position is critical for its biological activity. Further studies on the relationship between structure and activity are in progress.

References and Notes

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6. Brooks, D. W.; Lu, L. D.-L., Masamune, S. *Angew. Chem. Int. Ed. Engl.* **1979**, *18,* 72-74.
7. Selected spectral data of synthetic (*R*)-**1a** : ^{1}H-NMR (400 MHz, CD_3OD) δ 4.73 (dd, *J* = 3.2, 2.7 Hz, 1H), 3.95 (dd, *J* = 12.6, 2.7 Hz, 1H), 3.88 (dd, *J* = 12.6, 3.2 Hz, 1H), 2.87 (t, *J* = 7.6 Hz, 2H), 1.66 (m, 2H), 1.25-1.50 (m, 24H), 0.90 (t, *J* = 6.9 Hz, 3H), IR (KBr) 3400, 2925, 2850, 1750, 1665, 1610 cm^{-1}, MS (EI) m/z 368 (M^+). We also prepared the sodium ($[\alpha]_D^{20}$ +58.5° (*c* 0.42, $CHCl_3$), mp 220-230 °C, dec.) and magnesium salt ($[\alpha]_D^{20}$ +161.3° (*c* 0.19, $CHCl_3$), mp 245-260 °C, dec.) of (*R*)-**1a** by treatment of (*R*)-**1a** with either 1 equiv of sodium methoxide in methanol or 1/2 mol equiv of magnesium ethoxide in THF. The spectra of these salts were, again, inconsistent with that of natural sample of RK-682.
8. Selected spectral data of the complex of (*R*)-**1a** : ^{1}H-NMR (400 MHz, CD_3OD) δ 4.40 (dd, *J* = 4.2, 2.7 Hz, 1H), 3.90 (dd, *J* = 12.3, 2.7 Hz, 1H), 3.81 (dd, *J* = 12.3, 4.2 Hz, 1H), 2.79 (t, *J* = 7.6 Hz, 2H), 1.60 (m, 2H), 1.25-1.50 (m, 24H), 0.90 (t, *J* = 6.9 Hz, 3H), IR (neat) 3400, 2925, 2855, 1730, 1635, 1560, 1470, 1040-1100 (broad) cm^{-1}, Elemental Analysis C 59.77, H 8.96. Silicon derivatives of β-diketone have been reported, see: (a) Rochow, E. G. "Comprehensive Inorganic Chemistry", Ed. Trotman-Dickenson, A. F. Vol. 1, pp1465-1467 (1973). (b) West, R. *J. Am. Chem. Soc.* **1959**, *80,* 3246-3249. They reported a 6-coordinated silicon complex such as $(acac)_3Si^+X^-$. In this case, however, other types of complexes such as (**1a** - $H)_2(OH)_2Si$ or its oligomers are also possible. Although the exact structure of this silicon complex is unclear, the biologically active form of RK-682 in the aqueous assay buffer should be (*R*)-**1a**, the hydrolyzed product of this complex.
9. The ability of a GST-VHR fusion protein to dephosphorylate *p*-nitrophenylphosphate was measured in a buffer containing 25 mM MOPS, pH 6.5, 5 mM EDTA, and 1 mM DTT. For VHR phosphatase, see: Ishibashi, T.; Bottaro, D. P.; Chan, A.; Miki, T.; Aaronson, S. A. *Proc. Natl. Acad. Sci. USA* **1992**, *89,* 12170-12174.
10. The ability of cdc25A and cdc25B to dephosphorylate *p*-nitrophenylphosphate was measured in a buffer containing 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 5 mM $MgCl_2$, 0.1 mM DTT, 0.1 mM PMSF, and 5% glycerol. For cdc25A, see: Hoffmann, I.; Draetta, G.; Karsenti, E. *EMBO J.* **1994**, *13,* 4302-4310. For cdc25B, see: Honda, R.; Ohba, Y. Nagata, A.; Okayama, H.; Yasuda, H. *FEBS Lett.* **1993**, *318,* 331-334.
11. The ability of rabbit PP1 to dephosphorylate *p*-nitrophenylphosphate was measured in a buffer containing 20 mM MOPS, pH 7.5, 60 mM 2-mercaptoethanol, 0.1 M NaCl, 1 mg/mL serum albumin, and 50% glycerol. The assays were carried out according to the UBI (Upstate Biotechnology Incorporated) method.

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