Note

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A Convenient Preparation of Bis(4-methoxyphenyl)methanethiol and Its Application in the Synthesis of Biotin Thioacid

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We have established a facile protocol for the preparation of bis(4-methoxyphenyl)methanethiol (**4**). Thiol **4** was used to prepare biotin thioester **7** which was later subjected to acidolysis to afford biotin thioacid (**1b**) in high yield. Compound **1b** represents a new biotinylating reagent worth exploring and its spectral data was reported for the first time.

Keywords: Thiol; Thioester; Thioacid; Biotin; Bis(4-methoxyphenyl)methyl.

INTRODUCTION

Biotin (**1a**), a coenzyme with extraordinarily high affinity for avidin/streptavidin, has been extensively utilized as a tag in biochemical research.[1] In our efforts to develop activity-based probes for various classes of enzymes, we have also frequently utilized a biotin group to serve as the key reporter group.[2] The unique binding interaction of biotin makes possible the detection as well as the enrichment of the biotinylated biomolecules from a complicated system.[3] The biontinylation step in a probe construction was mainly achieved by reacting an active ester of biotin, such as biotin-OSu, with a free amino group. We envisioned that increasing the repertoire of the biotinylating agents by exploiting a novel chemistry would offer great advantage and flexibility in the synthetic planning of the probes. A promising strategy involves the thioacid chemistry which has received much attention in recent years.[4] Due to their soft and powerful nucleophilic behavior, thioacids could selectively react with a broad range of functional groups, such as azides,[5] sulfonylazides,[6] and isonitriles.[7] These characteristic reaction features thus make biotin thioacid (**1b**) a potentially useful and attractive biotinylating agent. This viewpoint was also substantiated by a recent application of compound **1b** in "sulfo-click" ligation.[8] Despite its interesting application, compound **1b** has never been purified nor characterized previously. We herein describe a convenient procedure for the preparation and isolation of compound **1b**. We have also performed a detailed spectral characterization of the compound.

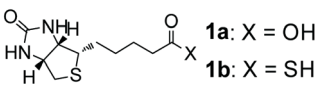

Fig. 1. The structures of biotin (**1a**) and biotin thioacid (**1b**).

Amongst the available thioacid acquiring methods,[9-13] the most commonly used approach in laboratory operation utilizes the thioester precursors because it does not require the use of toxic H_2S reagent and offers the advantage that these precursors could be easily obtained in a pure form in large quantity. In this approach, thiols were first coupled to the carboxylic acids and the resultant thioesters were subjected to deprotection to release the corresponding thioacids. Although thiols such as HSFmoc (**2**)[12] and HSTmob (**3**)[13] were commonly used in the preparation of thioesters, these thiols were not readily accessible (Figure 2). In our search for a convenient alternative, we found that 4,4′-dimethoxybenzhydryl group was previously reported as a protecting group for the thiol functionality of cysteine through a thioether linkage.[14] In the present study, we further explored this protecting group and established a facile protocol for the preparation of bis(4-methoxyphenyl)methanethiol (**4**), which will serve as a reliable thiol source for thioester formation.

RESULTS AND DISCUSSION

As shown in Scheme 1, commercially available bis(4-methoxyphenyl)methanol (**5**) was easily converted to thioacetate **6** by reacting it with thioacetic acid in the pres-

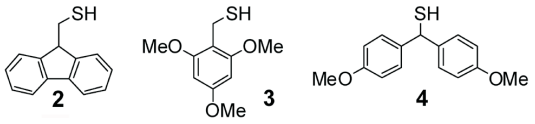

Fig. 2. The structures of thiols **2** (HSFmoc), **3** (HSTmob), and **4**.

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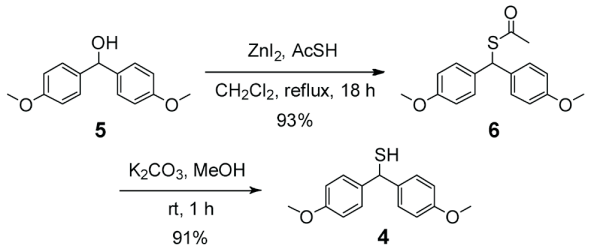
Scheme 1 Synthesis of bis(4-methoxyphenyl)methanethiol (**4**)



ence of ZnI_2 in CH_2Cl_2.[15] A simple aqueous workup and extraction procedure gave compound **6** in high yield (93%). This procedure could be readily applied to a multigram-scale preparation of compound **6** without the need for tedious purification steps. Thioacetate **6** served two purposes: firstly, it was used to generate thiol **4** by treatment with K_2CO_3 in MeOH under argon atmosphere. Although it was known that a free thiol group might undergo slow oxidation to a disulfide or a sulfoxide under ambient conditions,[16] we found that the quality of thiol **4** could be maintained over a long period of time when it was frozen in benzene solution.

Secondly, thioacetate **6** was used as a model to investigate the optimal conditions for thioacid formation under acidolysis with TFA (Figure 3). We performed time course experiments by incubating **6** (0.1 M) with varying concentrations of TFA (5%, 10%, 20%, 40%) and 8 equivalents of Et_3SiH in $CDCl_3$. The progress of the acidolysis was monitored by ^{1}H-NMR spectroscopy and the extent of the reaction was calculated from the integration of the signals in the aromatic region of the residual **6** and the bis(4-methoxyphenyl)methane byproduct (**8**). The preliminary data revealed that TFA concentrations higher than 20% were effective for the release of thioacid.

Thiol **4** was then coupled with biotin-OSu in DMF under an atmosphere of Ar at room temperature overnight to afford the biotin thioester **7** in 82% yield (Scheme 2). Although formation of a trace amount of disulfide was ob-

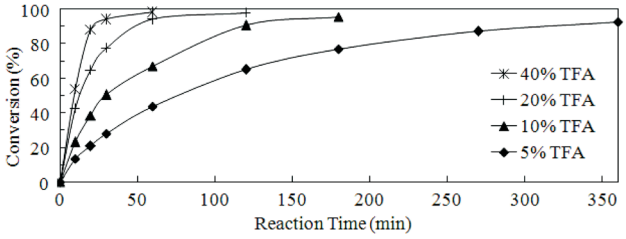
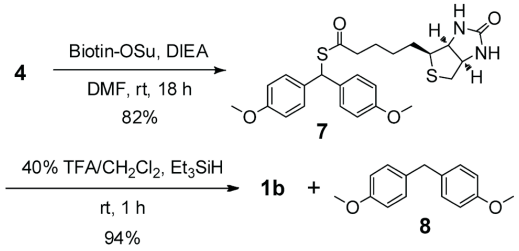


Fig. 3. Time-course acidolysis of thioacetate **6** with TFA for thioacid formation.

Scheme 2 Synthesis of biotin thioacid (**1b**)



served, it was easily separated and removed by silica gel column chromatography. Finally, the thioester **7** was subjected to acidolysis under conditions that were previously optimized (20-40% TFA) to release the biotin thioacid (**1b**). The nonpolar byproduct **8** formed in the acidolysis was conveniently removed by repeatedly washing the mixture with ether. Compound **1b** was thus obtained in high yield (94%) without any hydrolyzed contaminant. More importantly, compound **1b** could be stored as a suspension in *n*-hexane for a long period of time. We observed that the purity of compound **1b** remained greater than 95% even after 3 months of storage. This application demonstrates that thiol **4** could be regarded as a useful synthetic equivalent of hydrosulfide. It is interesting to note that the ^{1}H-NMR spectrum of compound **1b** resembles that of biotin, except the α-methylene group of compound **1b** displayed a dramatic downfield shift, from δ 2.20 ppm to 2.65 ppm (Figure 4). A similar downfield shift phenomenon was also observed for the thiocarboxylic group, from δ 175.0 ppm to 193.7 ppm, in the ^{13}C-NMR spectra.[17] In addition, compound **1b** showed an absorption band at 2531 cm^{-1} in the IR spectrum, which was assigned to the characteristic S-H stretching.[17,18]

CONCLUSIONS

In summary, we have established a facile protocol for the preparation of bis(4-methoxyphenyl)methanethiol (**4**),

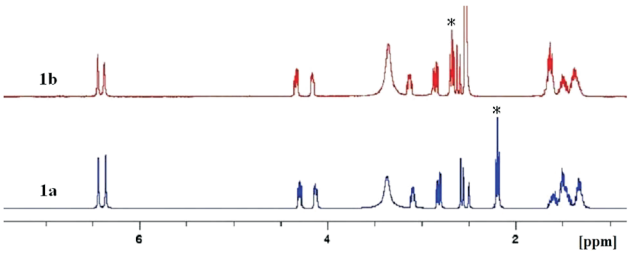


Fig. 4. ^{1}H-NMR spectra of biotin (**1a**) and biotin thioacid (**1b**) in DMSO-d_6. The asterisk denotes the signals of the α-methylene group.

which is a useful synthetic equivalent of hydrosulfide. Thiol **4** was used to prepare biotin thioester **7** which was later subjected to acidolysis to afford biotin thioacid (**1b**) in high yield. Compound **1b** represents a new biotinylating reagent worth exploring. The results of its applications will be described in due course.

EXPERIMENTAL

General Methods: All reagents and starting materials were obtained from commercial suppliers and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively. IR spectra were recorded at room temperature. The reactions were monitored by analytical TLC and spots were visualized under UV light and/or phosphomolybdic acid/ethanol stain. Column chromatography was performed with silica gel (230-400 mesh). Melting points are uncorrected.

***S*-4,4′-Dimethoxybenzhydryl Thioacetate (6):** To a solution of bis(4-methoxyphenyl)methanol (**5**, 3.00 g, 12.3 mmol) and thioacetic acid (961 μL, 13.5 mmol) in 150 mL of anhydrous CH_2Cl_2 was added ZnI_2 (2.00 g, 6.14 mmol) and the reaction mixture was refluxed for 18 h. Thereafter, the solution was allowed to cool to room temperature and then evaporated to dryness. The residue obtained was dissolved in EtOAc (200 mL) and the solution was washed with 5% $NaHCO_3$(aq) (30 mL ×3), H_2O (30 mL ×3) and brine (30 mL ×2). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. Without further purification, the desired product **6**[19] was obtained as a yellow oil in 93% yield. R_f = 0.52 (hexane/EtOAc = 1/1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.24 (d, *J* = 8.7 Hz, 4 H), 6.82 (d, *J* = 8.7 Hz, 4 H), 5.87 (s, 1 H), 3.76 (s, 6 H), 2.32 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 194.1 (C), 158.6 (C), 133.3 (C), 129.3 (CH), 113.8 (CH), 55.2 (CH), 50.7 (CH_3), 30.3 (CH_3). IR (neat): 3000, 2955, 2835, 1688, 1608, 1509, 1461, 1353, 1301, 1246, 1176, 1133, 1108, 1033, 955, 817, 778, 629, 585, 551 cm^{-1}. EI-MS *m/z* (%): 259 ([M-Ac]$^+$, 3), 227 ([M-SAc]$^+$, 100); HR-EI-MS calcd for $C_{17}H_{18}O_3S$ (M^+) 302.0977, found 302.0980.

Bis(4-methoxyphenyl)methanethiol (4): To a solution of compound **6** (116 mg, 0.38 mmol) in 4 mL of MeOH was added K_2CO_3 (159 mg, 1.15 mmol). The reaction mixture was purged and filled with Ar, stirred for 1 h at room temperature. It was then filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (eluent: 90% hexane/EtOAc) provided thiol **4**[20] as a colorless oil in 91% yield. R_f = 0.64 (hexane/EtOAc = 65/35). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 4 H), 6.87 (m, 4 H), 5.38 (d, *J* = 4.7 Hz, 1 H), 3.78 (s, 6 H), 2.22 (d, *J* = 4.7 Hz, 1 H, SH; ^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.5 (C), 135.8 (C), 128.7 (CH), 113.8 (CH), 55.2 (CH_3), 46.6 (CH). IR (neat): 3442, 2999, 2953, 2930, 2834, 2552 (SH), 2055, 1886, 1606, 1506, 1460, 1300, 1243, 1168, 1108, 1029, 810, 774, 626, 580 cm^{-1}. ESI-MS *m/z* (%): 259 ([M-H]$^-$, 100), 227 ([M-SH]$^-$, 17); HR-ESI-MS calcd for $C_{15}H_{15}O_2S$ $(M-H)^-$ 259.0793, found 259.0797.

Biotin Thioester (7): To a solution of compound **4** (258 mg, 0.99 mmol) and biotin-OSu (647 mg, 0.99 mmol) in DMF (10 mL) was added DIEA (246 μL, 1.49 mmol). The reaction mixture was purged and filled with Ar, and was stirred for 18 h at room temperature. The volatiles were removed in vacuo, and the residue obtained was dissolved in EtOAc (100 mL). The organic solution was washed with 5% $NaHCO_3$(aq) (30 mL, ×3), H_2O (30 mL, ×3), and brine (30 mL, ×2). The organic phase was then dried over anhydrous Na_2SO_4, filtered and concentrated. Purification by silica gel column chromatography (eluent: 90% $CHCl_3$/MeOH) provided the desired product **7** as a pale yellow oil in 82% yield. R_f = 0.22 ($CHCl_3$/MeOH = 9/1). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.21 (m, 4 H), 6.80 (m, 4 H), 5.83 (s, 1 H), 5.74 (s, 1 H, NH), 5.21 (s, 1 H, NH), 4.43 (m, 1 H), 4.20 (m, 1 H), 3.75 (s, 6 H), 3.06 (m, 1 H), 2.83 (dd, *J* = 12.8, 5.0 Hz, 1 H), 2.64 (d, *J* = 12.8 Hz, 1 H), 2.55 (t, *J* = 7.9 Hz, 2 H), 1.75-1.53 (m, 4 H), 1.46-1.28 (m, 2 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 197.8 (C), 158.6 (C), 133.3 (C), 133.3 (C), 129.3 (CH), 113.9 (CH), 61.9 (CH), 60.1 (CH), 55.2 (CH), 55.2 (CH), 50.5 (CH_3), 43.3 (CH_2), 40.5 (CH_2), 29.7 (CH_2), 28.1 (CH_2), 25.3 (CH_2). IR (neat): 3248, 2931, 1702, 1608, 1510, 1461, 1302, 1250, 1176, 1111, 817, 763, 587, 552 cm^{-1}. ESI-MS *m/z* (%): 509 ([M+Na]$^+$, 100), 261 (8), 229 (17); HR-ESI-MS calcd for $C_{25}H_{30}NaN_2O_4S_2$ $(M+Na)^+$ 509.1545, found 509.1545.

Biotin Thioacid (1b): To a solution of thioester **7** (100 mg, 0.20 mmole) in 2 mL of 40% TFA/CH_2Cl_2 was added Et_3SiH (266 μL, 1.60 mmol). The reaction mixture was stirred for 1 h at room temperature under an Ar atmosphere. The volatiles were then removed in vacuo, and the residue was dissolved in a minimum amount of $CHCl_3$. The solution was diluted with ether until precipitation occurred. The ether layer was removed with a dropper and the process was repeated for several times to afford the analytically pure **1b** as a white solid in 94% yield. R_f = 0.36 ($CHCl_3$/MeOH = 9/1), mp: 170-172 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.42 (s, 1 H, NH), 6.35 (s, 1 H, NH), 4.30 (m, 1 H), 4.13 (m, 1 H), 3.10 (m, 1 H), 2.82 (dd, *J* = 12.6, 5.1 Hz, 1 H), 2.65 (t, *J* = 7.5 Hz, 2 H), 2.58 (d, *J* = 12.6 Hz, 1 H), 1.70-1.27 (m, 6 H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 193.8 (C), 163.2 (C), 61.5 (CH), 59.7 (CH), 55.7 (CH), 42.4 (CH_2), 40.2 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 25.4 (CH_2). IR (KBr): 3250, 2916, 2531 (SH), 1703, 1671, 1476, 1397, 1166, 1082, 1022, 745, 663, 615 cm^{-1}. ESI-MS *m/z* (%): 259 ([M-H]$^-$, 100); HR-ESI-MS calcd for $C_{10}H_{15}N_2O_2S_2$ $(M-H)^-$

259.0575, found 259.0570.

^{1}H-NMR Time Course Acidolysis of Thioacetate 6: To solutions of compound **6** (30.2 mg, 0.10 mmol) in an array of TFA/$CDCl_3$ (5%, 10%, 20%, 40% TFA, and the total volume was 1 mL) were added Et_3SiH (129 μL, 0.80 mmol). The reaction mixtures were monitored with ^{1}H-NMR at designated intervals. The percent conversion for the reaction was calculated from the integration of the signals in the aromatic region of the residual **6** and the bis-(4-methoxyphenyl)methane byproduct (**8**).

ACKNOWLEDGEMENT

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SUPPORTING INFORMATION AVAILABLE:

^{1}H/^{13}C NMR spectra for compounds **6**, **4**, **7**, **1a**, **1b**, and **8** are included. IR and MS spectra for compound **1b** are also included.

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