Synthesis of novel ginkgolide photoaffinity–biotin probes†

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Site-selective introduction of biotin and benzophenone probes onto ginkgolide scaffolds is described.

Ginkgo biloba extract has been the focus of many biological studies, and ranks among the best selling herbal dietary supplements.[1] A number of accounts have indicated that minor congeners of the extract, namely polyoxygenated diterpenoids, collectively called ginkgolides or terpene trilactones (Fig. 1), are the principal components responsible for the physiological effects of the extract, including improvement of peripheral vascular function, inhibition of thrombosis and embolism,[2] as well as neuroprotection against amyloid peptide insults[3] (hence there is a potential application for the treatment of Alzheimer's disease and other amyloid-related cognitive disorders). However, the mode of ginkgolides' action with biological receptors has yet to be established on a molecular structural basis.

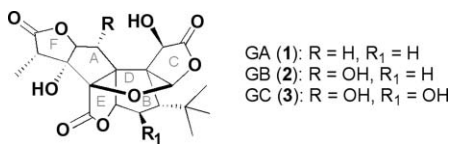

Fig. 1 Major ginkgolides from *Ginkgo biloba* leaf extract.

Antagonistic activity of ginkgolides towards several CNS-receptors, including platelet-activating factor receptor (PAFR),[4] glycine receptor,[5,6] and GABA receptor,[6] has been demonstrated recently. Furthermore, a preliminary biodistribution study of ^{18}F-labeled ginkgolide employing microPET techniques demonstrated the potential of ginkgolides to reach the brain.[7] The preparation of ginkgolide-bearing photoaffinity probes was also reported.[8] To facilitate the photocrosslinking approaches, we wish to report here on the synthesis of a new set of biotin-containing photoaffinity probes of GA (**1**) and GB (**2**). Both of these ginkgolides possess diverse biological activities, and the incorporation of biotin and photoaffinity probes onto these scaffolds is expected to reveal the mechanism of ginkgolide–receptor interactions.

Despite the fact that multifunctional probes have been introduced onto other natural products and synthetic compounds,[9] the unique structural and functional complexity of ginkgolides, *i.e.*, the rigid, cage-like skeleton as well as multiple hydroxy and lactone groups, presents a challenge for derivatization. Arguably, the structural and functional features of ginkgolides can also account for their various bioactivities. The importance of several structural elements was primarily demonstrated by the structure–activity studies using PAFR[10] and glycine receptor.[5] On the basis of those studies, two design strategies were envisioned (Fig. 2): (i) incorporation of a single, chimeric biotinylated photoaffinity probe *via* functionalization of the 10-hydroxy group, and (ii) ginkgolide functionalization with two distinct labels at different sites, *e.g.*, a benzophenone group *via* 10-OH and a terminal biotin moiety attached *via* C-15, following alteration of the F-lactone ring.

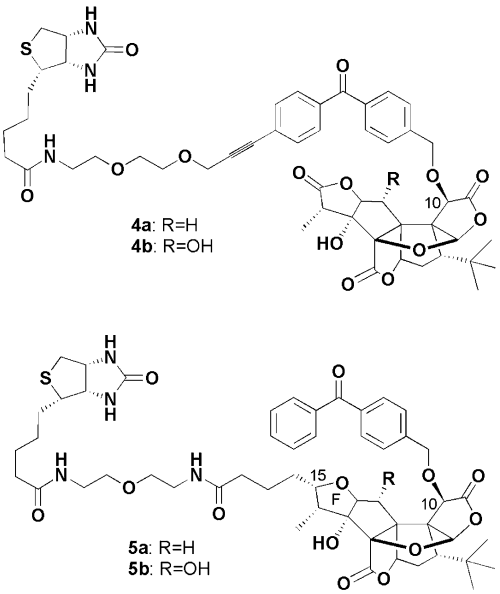

Fig. 2 Biotinylated photoaffinity-labeled ginkgolides.

We envisioned that the synthetic approaches should be modular in nature, thus allowing for facile fine-tuning of the ginkgolide-based probes for studying the interactions with a particular receptor. Furthermore, assembling the desired compounds on the ginkgolide scaffold (rather than first synthesizing the biotin and benzophenone probes and introducing them onto the ginkgolides as the last step) will create a number of ginkgolide derivatives that can be useful for subsequent structure–activity studies, and it will also allow us to probe the robustness of ginkgolide functionalities towards several synthetic transformations.

Synthesis of probes **4a** and **4b** was done according to Scheme 1. In both cases, the desired probes were obtained in three steps from the native ginkgolides. Preparation of GA-based probe **4a** is representative: selective modification of GA (**1**) at the 10-OH position with a benzophenone **6** was readily carried out using KH

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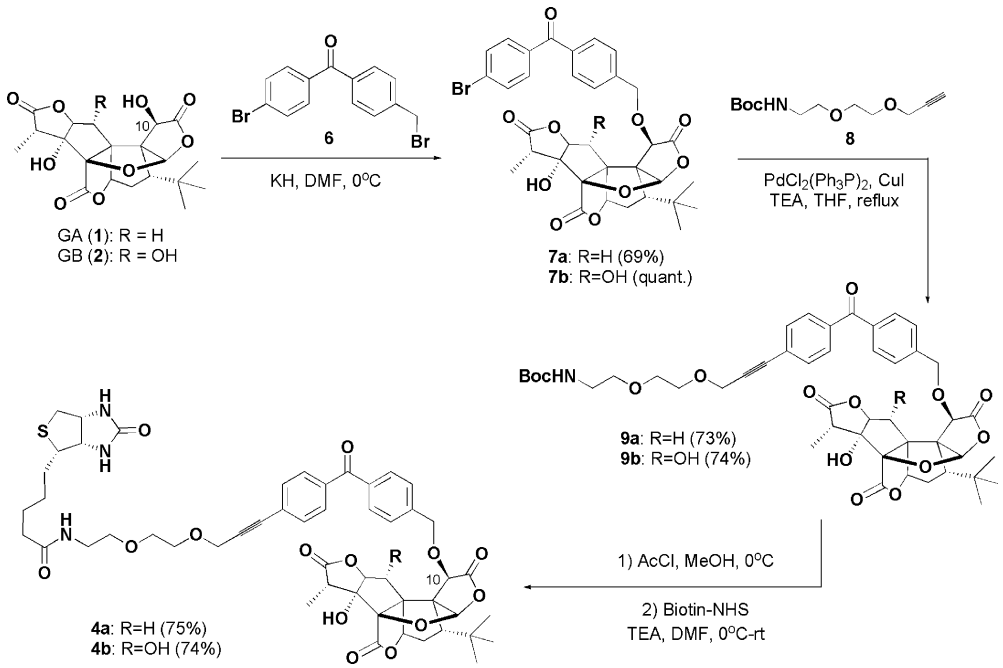
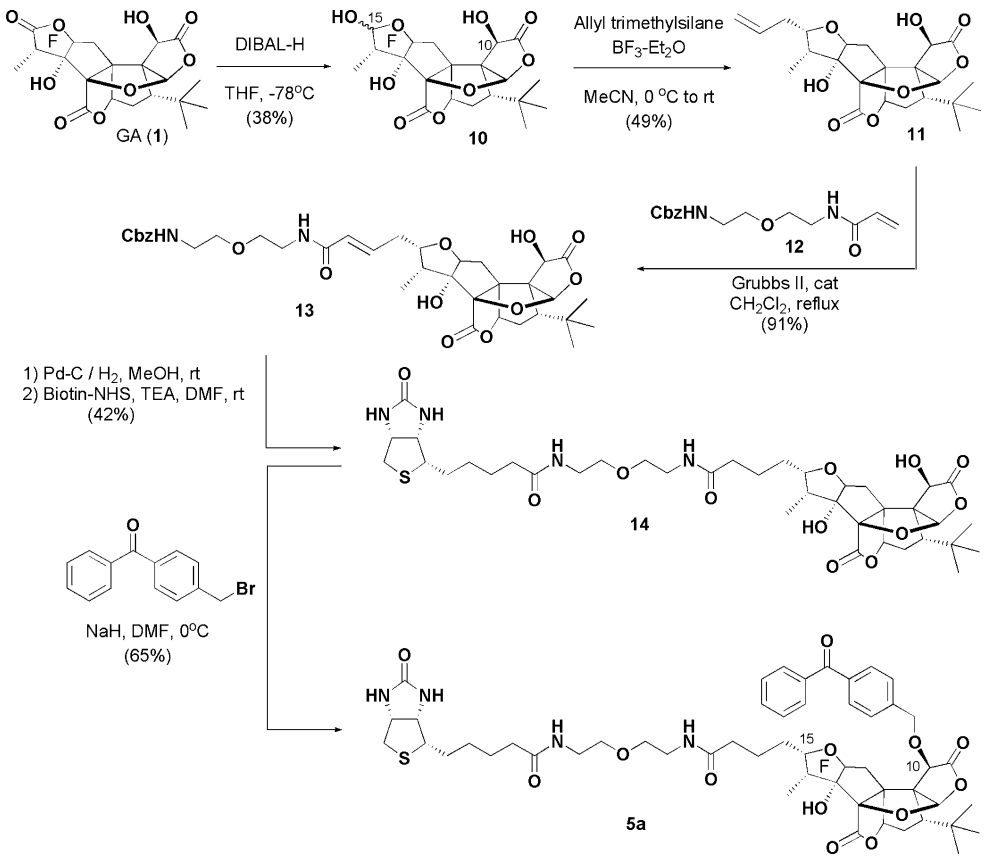


Scheme 1 Synthesis of probes **4a** and **4b**.

as a base. Compound **7a**[11] was coupled to **8** under Sonogashira conditions; removal of the Boc-protecting group furnished the amine that was directly reacted with *N*-hydroxysuccinimide biotin (biotin-NHS) to give the desired probe **4a**.

Introduction of the biotin-based probe *via* modification of the ginkgolide skeleton required some alteration of the synthetic route depending on the identity of the ginkgolide scaffold (Scheme 2), unlike the approach outlined in Scheme 1. Since the selective



Scheme 2 Synthesis of **5a**.

reduction of F-lactone was reported previously,[12] we started with DIBAL-H reduction of GA (**1**) to obtain **10** as a mixture of epimers, which was not separated, but directly subjected to the reaction with allyl trimethylsilane to afford **11** and epi-**11** as a 9 : 1 mixture. Pure **11** was readily isolated by column chromatography,[13] and cross coupled with the protected amine **12** using Grubbs' second generation catalyst. The obtained precursor **13** was then subjected to hydrogenolysis to remove the Cbz group and the deprotected amine was directly reacted with biotin-NHS leading to biotinylated product **14** in moderate yield. Finally, the benzophenone moiety was attached *via* the 10-hydroxy group, thus furnishing the desired probe **5a**.

Due to availability of 10-BnGB (**15**), which is a mandatory intermediate during isolation and purification of ginkgolides,[14] and the fact that Cbz and Bn groups can be removed under identical conditions, we decided to use **15** as the starting material (instead of **2**) for the synthesis of GB-based probe **5b** (Scheme 3). Reduction of the ring-F yielded lactol **16** as a mixture of epimers, which was not separated and was directly used for subsequent transformations. Unexpectedly, and in sharp contrast to the GA-case (Scheme 2), the subsequent reaction with allyl trimethylsilane failed to give the desired allylated ginkgolide and unchanged **15** was recovered as the only product. Assuming that a hydrogen bonding between 1-OH (which is absent in GA) and the ring-F ethereal oxygen might be responsible for observed unreactivity of **16**, the lactol hydroxyl group was acetylated, and **17** was then subjected to allylation reaction giving **18** as a single epimer in a moderate yield.[13] Apparently, the observed selectivity can be attributed to the "blocking" effect of the Bn-group. The allylated ginkgolide **18** was further reacted with **12** *via* olefin cross metathesis protocol to yield **19**. Subsequent hydrogenolysis removed both Cbz and Bn groups and elaborated the amine, which was readily coupled with biotin-NHS to give a biotinylated ginkgolide **20**. Although debenzylation under Pd/C–H_2 conditions usually requires high pressure, we found that addition of molecular sieves to the reaction mixture allowed a room-temperature deprotection to take place. Noteworthy, under these conditions GB (**2**) was cleanly obtained from **15** (see ESI†). Finally, the treatment of **20** with 4-bromomethylbenzophenone in the presence of K_2CO_3 selectively introduced the benzophenone moiety at the 10-position, yielding **5b**.

Thus, new types of ginkgolide-based biotin–photoaffinity probes, which should facilitate efforts for establishing ginkgolide–receptor interactions, and potentially can be used for discovery of novel ginkgolide-binding receptors, have been synthesized.‡

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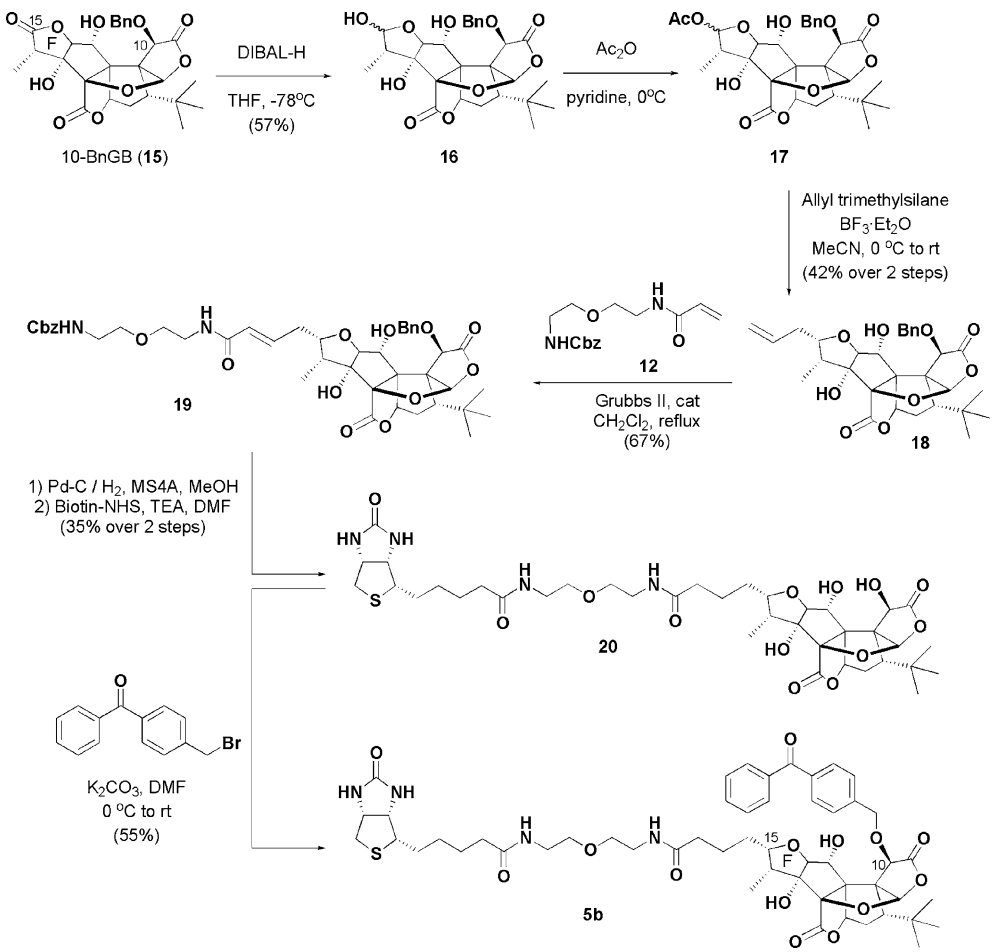

Scheme 3 Synthesis of **5b**.

Notes and references

‡ **4a**. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.81$ (2H, d, $J = 8.2$ Hz), 7.80 (2H, d, $J = 7.8$ Hz), 7.61 (1H, t, $J = 7.4$ Hz), 7.50 (2H, dd, $J = 7.4$, 7.8 Hz), 7.41 (2H, d, $J = 8.2$ Hz), 6.78 (1H, t, $J = 5.4$ Hz, –NH), 6.71 (1H, t, $J = 5.2$ Hz, –NH), 6.17 (1H, s, –NH), 5.98 (1H, s), 5.57 (1H, d, $J = 11.2$ Hz), 5.45 (1H, s, –NH), 4.81 (1H, s), 4.69 (1H, d, $J = 4.0$ Hz), 4.61 (1H, d, $J = 11.2$ Hz), 4.53–4.48 (2H, m), 4.36 (1H, dt, $J = 2.8$, 10.6 Hz), 4.30 (1H, dd, $J = 4.8$, 7.2 Hz), 4.15 (1H, s, –OH), 3.55–3.51 (4H, m), 3.46–3.34 (4H, m), 3.13 (1H, dt, $J = 4.8$, 7.2 Hz), 2.91 (1H, dq, $J = 7.1$, 7.1 Hz), 2.89 (1H, dd, $J = 4.8$, 12.8 Hz), 2.71 (1H, d, $J = 12.8$ Hz), 2.42 (1H, dd, $J = 7.0$, 15.0 Hz), 2.29–2.20 (4H, m), 2.17 (1H, dd, $J = 4.8$, 13.8 Hz), 2.11 (1H, dd, $J = 8.4$, 15.0), 2.08 (1H, ddd, $J = 4.0$, 13.8, 13.8 Hz), 1.92 (1H, dd, $J = 4.8$, 13.8 Hz), 1.75–1.59 (8H, m), 1.43 (2H, tt, $J = 7.5$, 7.5 Hz), 1.09 (9H, s), 1.06 (3H, d, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 196.22$, 173.81, 173.66, 172.97, 172.16, 163.79, 140.91, 137.47, 137.35, 132.63, 130.38, 130.03, 128.38, 127.40, 110.12, 101.77, 89.82, 89.81, 86.67, 84.45, 76.05, 71.95, 69.84, 69.57, 69.15, 66.85, 63.72, 61.78, 60.18, 55.46, 48.94, 40.52, 39.12, 38.96, 38.13, 36.47, 36.36, 35.52, 32.24, 31.22, 29.19, 27.93, 27.84, 25.43, 22.98, 7.88; HRMS (FAB+): $[M + H]^+$ m/z calcd for $C_{52}H_{67}O_{13}N_4S$ 987.4425, found 987.4427. **4b**. ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.82$ (2H, d, $J = 8.0$ Hz), 7.77 (2H, d, $J = 8.0$ Hz), 7.66 (1H, t, $J = 8.0$ Hz), 7.59 (2H, d, $J = 8.0$ Hz), 7.54 (2H, t, $J = 8.0$ Hz), 6.10 (1H, s), 5.57 (1H, d, $J = 11.0$ Hz), 5.29 (1H, d, $J = 4.0$ Hz), 5.26 (1H, s), 4.85 (1H, d, $J = 11.0$ Hz), 4.47 (1H, dd, $J = 5.0$, 7.3 Hz), 4.29–4.25 (2H, m), 4.23 (1H, d, $J = 7.0$ Hz), 4.19 (1H, d, $J = 7.0$ Hz), 3.55–3.45 (4H, m), 3.35–3.30 (4H, m), 3.19 (1H, dt, $J = 4.2$, 9.0 Hz), 2.91 (1H, dd, $J = 5.0$, 12.8 Hz), 2.79 (1H, dq, $J = 3.0$, 7.0 Hz), 2.68 (1H, d, $J = 12.8$ Hz), 2.28–2.19 (5H, m), 2.00 (1H, ddd, $J = 4.0$, 4.0, 13.8 Hz), 1.89 (1H, dd, $J = 4.0$, 14.3 Hz), 1.74–1.53 (8H, m), 1.42 (2H, tt, $J = 7.5$, 7.5 Hz), 1.14 (9H, s), 1.04 (3H, d, $J = 7.0$ Hz); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 197.99$, 176.39, 176.24, 173.90, 173.86, 166.10, 142.35, 139.04, 138.69, 133.96, 131.63, 131.03, 129.60, 129.33, 111.81, 101.58, 96.06, 87.20, 85.47, 85.35, 80.59, 77.88, 76.02, 73.95, 73.70, 70.48, 70.44, 68.69, 63.37, 61.64, 57.00, 50.63, 43.19, 41.06, 40.30, 40.25, 38.24, 36.81, 36.76, 33.16, 32.64, 29.75, 29.51, 26.84, 24.14, 8.21; HRMS (FAB+): $[M + H]^+$ m/z calcd for $C_{52}H_{67}O_{14}N_4S$ 1003.4375, found 1003.4391. **5a**. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.79$ (2H, d, $J = 8.2$ Hz), 7.77 (2H, d, $J = 8.4$ Hz), 7.55 (2H, d, $J = 8.4$ Hz), 7.42 (2H, d, $J = 8.2$ Hz), 6.72 (1H, t, $J = 5.5$ Hz, –NH), 6.40 (1H, s, –NH), 5.99 (1H, s), 5.70 (1H, s, –NH), 5.55 (1H, d, $J = 11.0$ Hz), 4.86 (1H, s), 4.83 (1H, dd, $J = 7.8$, 7.8 Hz), 4.76 (1H, d, $J = 3.6$ Hz), 4.63 (1H, d, $J = 11.0$ Hz), 4.49 (1H, dd, $J = 4.8$, 7.4 Hz), 4.45 (2H, s), 4.28 (1H, dd, $J = 4.6$, 7.4 Hz), 3.75 (2H, m), 3.69 (2H, m), 3.59 (2H, t, $J = 5.0$ Hz), 3.43 (2H, dt, $J = 5.5$, 5.0 Hz), 3.16 (1H, q, $J = 7.2$ Hz), 3.10 (1H, dt, $J = 4.6$, 7.2 Hz), 2.87 (1H, dd, $J = 4.8$, 12.8 Hz), 2.72 (1H, dd, $J = 7.8$, 15.2 Hz), 2.71 (1H, d, $J = 12.8$ Hz), 2.23–2.17 (4H, m), 2.11 (1H, ddd, $J = 3.6$, 13.6, 13.6 Hz), 1.95 (1H, dd, $J = 4.8$, 13.6 Hz), 1.75–1.55 (4H, m), 1.39 (2H, t, $J = 7.4$ Hz), 1.31 (3H, d, $J = 7.2$ Hz), 1.10 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 195.52$, 176.67, 173.66, 171.74, 171.70, 164.23, 141.14, 137.28, 137.02, 131.79, 130.53, 130.17, 127.73, 126.97, 110.15, 101.18, 88.31, 87.55, 86.82, 86.52, 85.84, 75.96, 72.17, 70.06, 70.00, 69.38, 69.05, 67.36, 61.91, 60.39, 59.29, 55.71, 49.05, 40.94, 40.65, 39.26, 37.38, 36.73, 36.07, 32.40, 29.30, 28.36, 28.17, 25.68, 8.20; HRMS (FAB+): $[M + H]^+$ m/z calcd for $C_{51}H_{60}O_{14}N_3S$ 970.3796, found 970.3824. **5b**. ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.80$ (2H, d, $J = 8.3$ Hz), 7.77 (2H, d, $J = 8.5$ Hz), 7.60 (2H, d, $J = 8.5$ Hz), 7.59 (2H, d, $J = 8.3$ Hz), 6.15 (1H, s), 5.58 (1H, d, $J = 11.5$ Hz), 5.40 (1H, d, $J = 4.0$ Hz), 5.27 (1H, s), 4.84 (1H, d, $J = 11.5$ Hz), 4.54 (1H, d, $J = 7.2$ Hz), 4.48 (2H, s), 4.47 (1H, dd, $J = 5.0$, 8.0 Hz), 4.30 (1H, d, $J = 7.2$ Hz), 4.28 (1H, dd, $J = 4.4$, 8.0 Hz), 3.77 (2H, m), 3.68 (2H, m), 3.57 (2H, t, $J = 5.5$ Hz), 3.38 (2H, t, $J = 5.5$ Hz), 3.17 (1H, dt, $J = 4.4$, 9.0 Hz), 3.05 (1H, q, $J = 7.0$ Hz), 2.90 (1H, dd, $J = 5.0$, 12.7 Hz), 2.68 (1H, d, $J = 12.7$ Hz), 2.26 (1H, dd, $J = 4.4$, 13.6 Hz), 2.21 (2H, t, $J = 7.5$ Hz), 2.06 (1H, ddd, $J = 4.0$, 13.6, 14.2 Hz), 1.91 (1H, dd, $J = 4.4$, 14.2 Hz), 1.76–1.53 (4H, m), 1.41 (2H, t, $J = 7.8$ Hz), 1.23 (3H, d, $J = 7.0$ Hz), 1.13 (9H, s); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 197.01$, 178.32, 176.16, 173.68, 172.51, 166.09, 142.80, 138.51, 138.32, 132.72, 131.47, 131.13, 129.06, 128.42, 111.84, 100.81, 94.15, 89.54, 86.32, 84.55, 80.47, 77.79, 75.49, 73.83, 73.62, 71.15, 70.65, 70.52, 70.51, 69.24, 63.36, 61.64, 59.79, 56.98, 50.66, 43.29, 41.05, 40.34, 38.27, 36.76, 33.18, 29.74, 29.45, 26.84, 8.23; HRMS (FAB+): $[M + H]^+$ m/z calcd for $C_{51}H_{60}O_{15}N_3S$ 986.3745, found 986.3780.

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11 **7** is a potentially useful intermediate for a variety of metal-catalyzed coupling reactions.

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13 The significance of this allylation stems from the fact that it provides a handle for subsequent transformations, while preserving the native structural integrity of the west part of the ginkgolide, which was shown to be crucial for receptors' binding affinities.

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