The Preparation of Gibberellin Hapten–protein Conjugates. Part 2.[1] Conjugates and Gibberellin Affinity Probes Formed *Via* the Addition of α,ω-Dithiols to C-16-enes

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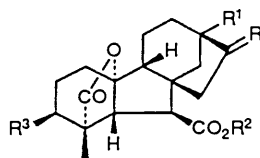
The free radical addition of α,ω-dithiols to a range of gibberellins is described. The resulting 17-mercaptoalkylthiogibberellins were easily coupled, *via* reaction with maleic anhydride, to protein carriers for use of as antigens in the preparation of anti-gibberellin antibodies. These derivatives were also allowed to react with a number of electrophilic reagents to prepare fluorescent, biotinylated and photoreactive gibberellin probes, as well as immobilised gibberellins.

In a previous paper a strategy for the synthesis of gibberellin (GA)–protein conjugates for use in the production of monclonal antibodies to GAs was described.[1] To obtain antibodies that discriminate between GAs having structural differences in ring-A, we needed to develop methods of conjugation at C-17 of the GA molecule. The first approach[1] was based on C-17-carboxylated GA_4 **1**, prepared by Peterson olefination of the 17-norgibberellin 16-ketone **2**, available from osmium tetraoxide–sodium periodate treatment of GA_4 **3**. However, this route is not readily applicable to a wide variety of GAs, many of which are not available in sufficient quantity for extended synthetic routes. The Peterson olefination reaction also fails for

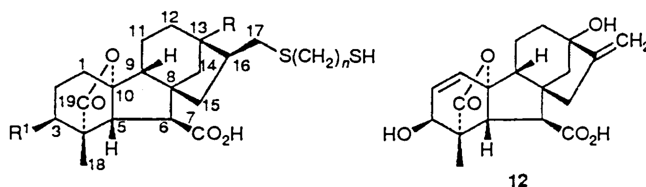


1; R = $CHCO_2H$, R^1 = H, R^2 = H, R^3 = OH
2; R = O, R^1 = H, R^2 = H, R^3 = OH
3; R = CH_2, R^1 = H, R^2 = H, R^3 = OH
4; R = CH_2, R^1 = H, R^2 = $SnBu_3$, R^3 = OH
5; R = CH_2, R^1 = OH, R^2 = H, R^3 = OH
6; R = CH_2, R^1 = H, R^2 = H, R^3 = H

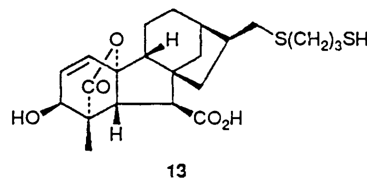
16-ketones derived from 13-hydroxy GAs such as GA_1 **5**, due, we believe, to increased steric hindrance.[2] In this present paper a simple one step method for the preparation of 17-mercaptoalkylthio-GAs is reported. This reaction can be applied to almost all of the 85 known gibberellins. The free thiol group and spacer-arm so introduced allowed specific coupling to a number of molecules, including antigenic proteins and affinity probes such as biotin and fluorescent molecules.

Results and Discussion

The reaction of gibberellins with α,ω-dithiols was optimised using GA_4 **3** as a representative gibberellin and propane-1,3-dithiol as a co-reactant. Treatment of a refluxing benzene solution of GA_4, containing 0.5 equiv. of bis(tributyltin) oxide, with a large excess of α,ω-dithiol and a catalytic amount of α-azoisobutyronitrile (AIBN) gave a high yield of the 1 : 1 coupling product **7**. The function of the bis(tributyltin) oxide was two-fold. Initially the gibberellin was solubilised by formation of the 7-tributyltin ester **4**. Also the presence of tributyltin oxide was shown to be essential for efficient addition to take place. In its absence the product **7** was formed slowly and full conversion of starting material was difficult to achieve. It is proposed that this enhancement is due to the formation of tributyltinthioethers (RSSnBu) which results in efficient propagation of the radical chain reaction. Indeed some support for the formation of this species arises from the observation than when the (soluble) tributyltin esters of the more polar gibberellins GA_1 **5** and GA_3 **12** in refluxing benzene or toluene were treated with thiols an immediate precipitation of GA_1 or GA_3 occurred. This indicated cleavage of the stannyl esters, presumably by nucleophilic attack of RSH on tin. As a consequence these gibberellins failed to yield addition products in these solvents. In a survey of other solvents, addition of α,ω-dithiols to the exocyclic methylene of **5** and **12** was found to occur in dioxane. Again, in the absence of tributyltin oxide, reactions were slow and incomplete after 12 h refluxing. However, the addition of 0.5 equiv. of the tin compound resulted in clean formation of **9** and **13** in high yield after 4 h.



7; n = 3, R = H, R^1 = OH
8; n = 6, R = H, R^1 = OH
9; n = 3, R = OH, R^1 = OH
10; n = 3, R = H, R^1 = H
11; n = 6, R = H, R^1 = H



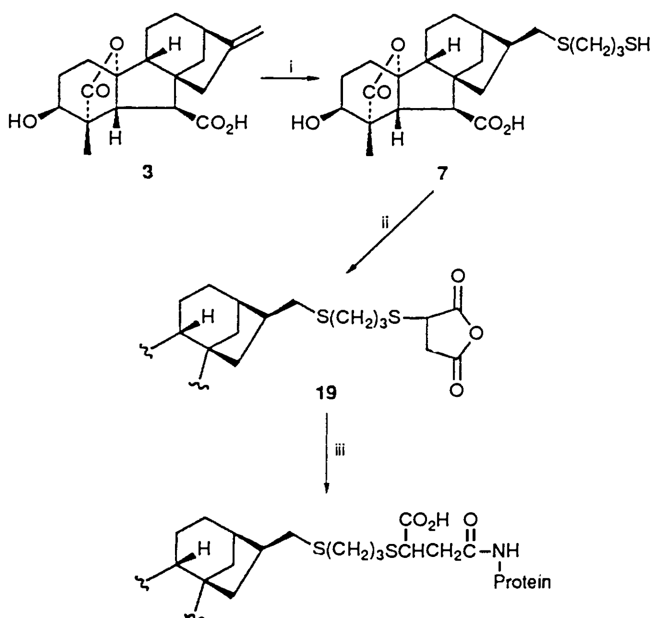
The identity of the 1 : 1 addition products was confirmed by mass spectrometry; prominent molecular ions were observed under electron impact ionisation and similar (M^+ − 1) ions under negative ion fast atom bombardment. The ^1H NMR spectra showed the absence of the ring D olefinic signals and the retention of the signals assigned to protons of rings A and B. It is noteworthy that the double bond of ring A of GA_3 **12** was not affected during this reaction. The presence of the three methylene groups adjacent to sulphur was indicated by a 6-H multiplet at δ 2.62 (in **7**). This was resolved by a 2D-COSY experiment into two triplets (2 and 4-CH_2) and a doublet (17-CH_2). This experiment also located the multiplet assigned to 3′-CH_2 at δ 1.86 and a broad signal due to 16-H at δ 2.25. We were unable to assign the stereochemistry at C-16 by this

technique but the compounds were essentially one epimer and it is assumed that addition occurred from the less hindered α-face to yield the β-stereochemistry depicted.

Once isolated and dessicated *in vacuo* to a solid foam these compounds were stable if stored at −20 °C. However, examination of solutions of **7** by TLC, after being left in air and light revealed a slow decomposition to a more polar compound, whose ^1H NMR spectrum was very similar to **7**. Although a full structural investigation was not conducted this compound is believed to be to be the dimeric disulphide.

The above conditions were amenable to the preparation of GA derivatives with different length spacer arms as demonstrated by the preparation of **8** from GA_4 **3** and hexane-1,6-dithiol and of **10** and **11** from GA_9 **6** and propane-1,3-dithiol and hexane-1,6-dithiol respectively. These compounds were also prepared in radioactive form from the appropriate [^3H]-labelled gibberellin.

In order to couple these derivatives to proteins for use as antigens, maleic anhydride served as a bifunctional reagent forming a bridge to protein amino groups (Scheme 1). Reaction



Scheme 1 *Reagents:* i, $HS(CH_2)_3SH$, $(Bu_3Sn)_2O$, AIBN; ii, maleic anhydride, Bu_3N; iii, Protein, pH 9.0, dioxane–H_2O

of **7** with maleic anhydride and tributylamine to dry dioxane gave the substituted succinic anhydride **19** whose ^1H NMR spectrum was difficult to interpret fully due to the introduction of the diastereoisotopic centre. However, the expected methine signal due to $SCHCO_2$ at δ 3.99 was observed as well as a small downfield shift of the triplets assigned to $4\text{-}CH_2S$ in the diastereoisomers. In the coupling reactions [^3H]-labelled **7**, **9** or **10** was allowed to react with maleic anhydride (TLC analysis) and the products were treated with aqueous solutions of proteins (keyhole limpet hemocyanin or bovine serum albumin) at pH 9.0. In this way aminolysis of the substituted succinic anhydride by protein lysyl residues resulted in good coupling ratios (*e.g.* GA_9 **6**:BSA, 17:1) as determined by [^3H]-radiocounting of dialysed and lyophilised protein products. Use of these conjugates to prepare monoclonal antibodies which are specific to the exposed rings A/B of the parent gibberellins has been described elsewhere.[3,4]

The mercaptoalkyl derivatives **7** and **8** of the biologically active gibberellin A_4 **3** were also exploited in the preparation of a number of molecular probes for potential GA-binding sites. The nucleophilicity of the terminal thiol group enabled a variety of coupling reactions to link probes specifically *via* the extended C-17, thereby retaining the biologically important functionalities of rings A and B. The coupling chemistries used are summarised in Scheme 2.

Fluorescein labelled-GA_4 **14** was prepared by reaction of **7** with fluorescein isothiocyanate. This compound gave the expected fluorescence spectra and was characterised by ^1H and ^{13}C NMR and mass spectrometry. For dansyl-labelled gibberellins the direct reaction of **7** with dansyl* chloride failed, the product being a polar dimeric derivative of **7** perhaps formed by displacement of the dansyl group of the originally formed *S*-dansyl **7** by the thiol group of another molecule of **7**. However, reaction of **7** with dansyl aziridine[5] cleanly afforded the dansylated compound **15**.

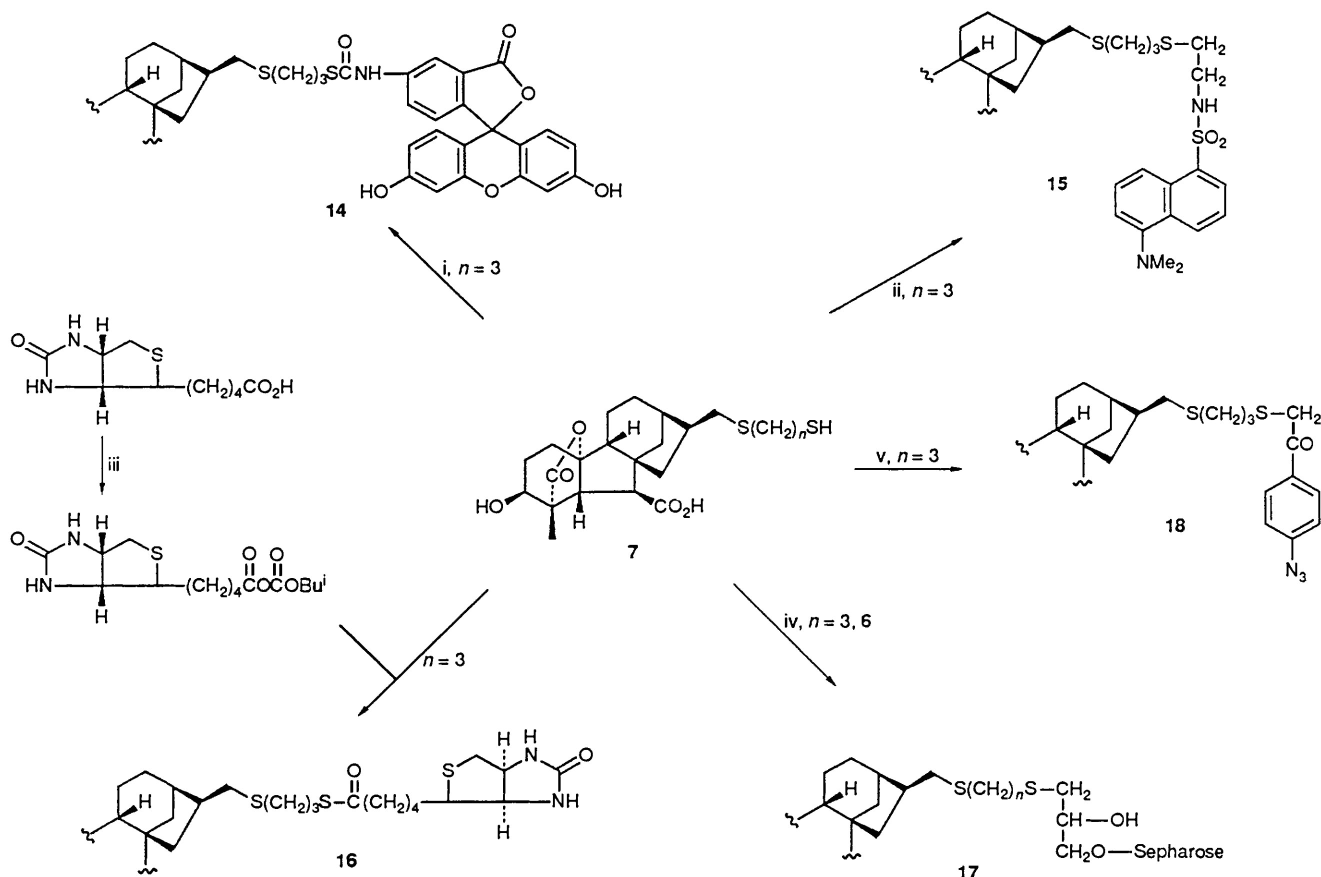
Prior to coupling to **7** biotin was activated by formation of a mixed anhydride with isobutylchloroformate. The activated biotin reacted smoothly with **7** to yield the thioester **16**. The immobilisation of bioactive gibberellins in such a way as to expose the essential functional groups for binding by gibberellin receptors and metabolic enzymes was an important goal in our research. Knofel *et al.*,[6] have described gibberellin affinity columns based on coupling to the 7-carboxy group of gibberellins. As this group is essential for gibberellin bioactivity it is unlikely that these adsorbents would be useful in GA-receptor research. The mercaptoalkylthio derivatives **7** and **8** described above, however, were readily coupled to epoxy-activated Sepharose 6B. This provided a means of specific coupling, as well as a method of varying the spacer-arm length. Sepharose 6B was activated by reaction with epichlorohydrin as described by Matsumoto *et al.*[7,8] Reaction of **7** and **8** with such material was confirmed and quantified by microanalysis, for sulphur, of dried matrix. These affinity matrices **17** have permitted the location of a putative gibberellin receptor to plasma membranes of plant cells.[9] The mercaptoalkylthio groups are also useful for the preparation of biologically active photoaffinity labels. This was demonstrated by the reaction of **7** with *p*-azidophenacyl bromide to yield the *S*-azidophenacyl derivative **18**. This compound is the first gibberellin photoaffinity label and has been shown to be biologically active and to photo-inactivate gibberellin-induced responses in aleurone protoplasts of *A. fatua*.[10] Further developments in the synthesis of ^3H and ^{125}I labelled gibberellin-aryl azide photoprobes will be the subject of another publication.

Experimental

General methods have been described elsewhere.[11] *N*-Dansylaziridine [N-(5-dimethylamino-1-naphthylsulphonyl)aziridine] was obtained from Sigma, and *p*-azidophenacyl bromide from Fluka. *J* Values are given in Hz throughout. Light petroleum is the fraction of b.p. 60–80 °C.

17-*Mercaptopropylthio*-3α,10-*dihydroxy*-20-*norgibberella*-7,19-*dioic Acid* 19,10-*Lactone* **7**.—A suspension of gibberellin A_4 **3** (800 mg, 2.4 mmol) in benzene (50 cm^3) with bis(tributyltin) oxide (640 mm^3, 1.20 mmol) was refluxed under N_2 (Dean and Stark) for 0.5 h. Propane-1,3-dithiol (2.5 cm^3, 25 mmol) was added *via* a dropping funnel and refluxing was continued while α-azoisobutyronitrile (AIBN) (15 mg) in benzene (20 cm^3) was added dropwise over 3 h. The solution was then cooled, diluted with more benzene and extracted with saturated aqueous sodium hydrogen carbonate. The hydrogen carbonate layer was then acidified with 5 mol dm^{-3} hydro-

* For convenience, 5-dimethylamino-1-naphthylsulphonvl is referred to as dansyl throughout.



Scheme 2 *Reagents:* i, Fluorescein isothiocyanate, Et_3N, CH_2Cl_2; ii, Dansylaziridine; iii, $ClCO_2Bu^i$, Bu_3N, acetone, DMF; iv, Sepharose-OCH_3CHCH_2O dioxane–H_2O, pH 9; v, *p*-Azidophenacyl bromide, Et_3N

chloric acid to pH 3 and the product recovered in ethyl acetate. The resulting oil was flash chromatographed on a 20 × 2 cm column eluted stepwise with the following percentages of ethyl acetate in light petroleum containing 0.5% acetic acid: 30 (100 cm^3), 40 (100 cm^3), 50 (100 cm^3), 60 (100 cm^3), 70 (100 cm^3) and 80% (100 cm^3) and collected in 26 fractions. Fractions 12–17 contained the *title compound* **7** (961 mg) as a gum (Found: M^+, 440.1694. $C_{22}H_{32}O_5S_2$ requires *M*, 440.1697) $\delta_H[(CD_3)_2CO$; 400 MHz, COSY] 1.08 (s, 18-CH_3), 1.86 (m, SCH_2CH_2CHSH), 2.25 (br, 16-H), 2.53 (d, *J* 10.5, 6-H), 2.62 (m, 3 × CH_2S), 3.17 (d, *J* 10.5, 5-H) and 3.72 (br d, *J* 2, 3-H); *m/z* 440 (M^+, 100%), 365 [M − $(CH_2)_3SH$, 40], 270 (46), 269 (26), 229 (19), 225 (14), 108 (40) and 91 (31).

17-*Mercaptohexylthio*-GA_4 **8** was prepared in the same way from GA_4 **3** (400 mg), bis(tributyltin) oxide (320 mm^3), hexane-1,6-dithiol (2.0 cm^3) and AIBN (10 mg). This yielded **8**, (436 mg) after chromatography as above. $\delta_H(CDCl_3$; 400 MHz) 1.17 (s, 18-CH_2), 2.27 (br, 16-H), 2.51 (m, 3 × CH_2S), 2.66 (d, *J* 10.5, 6-H), 3.13 (d, *J* 10.5, 5-H) and 3.85 (br s, 3-H).

Radiolabelled 17-*mercaptopropylthio*-GA_9 *and* -GA_4. These were prepared in the same way as above and illustrated for [15,17-^3H]-GA_9: [15,17-^3H]-GA_9 **6** (155 mg, 7.16 × 10^6 dpm) in benzene (20 cm^3) with bis(tributylin) oxide (133 mm^3) and propane-1,3-dithiol (1 cm^3) and AIBN (7 mg). After work-up as above the product was purified by flash chromatography using ethyl acetate (0–40%) in light petroleum containing 0.5% acetic acid. Seventeen fractions were collected. Fractions 10–13 contained starting [^3H]-GA_9 (5 mg, 1 × 10^5 dpm) and fractions 14–16 yielded the required [^3H]-17-*mercaptopropylthio*-GA_9 **10** (141 mg, 3.96 × 10^6 dpm) (Found: M^+, 424.1693. $C_{22}H_{32}O_4S_2$ requires *M*, 424.1742) $\delta_H[(CD_3)_2CO]$ 1.02 (s, 18-Me), 2.51 (d, *J* 10.5, 5-H), 2.54 (d, *J* 10.5, 6-H), 2.63 (m, 3 × CH_2S); *m/z* 424 (M^+, 100%), 380 (15), 349 [M − $(CH_2)_3SH$, 42], 272 (612), 229 (33) and 185 (31).

17-*Mercaptopropylthio*-GA_3 **13**.—Gibberellin A_3 **12** (310 mg) in dry dioxane (10 cm^3) was treated with bis(tributyltin) oxide (228 mm^3). The solution was heated to reflux and then propane-1,3-dithiol (1.0 cm^3) was added with AIBN (5 mg). Refluxing under N_2 was continued for 4 h. The solution was then poured into saturated aqueous sodium hydrogen carbonate. The aqueous layer was washed with ethyl acetate and then acidified to pH 3.0 with conc. hydrochloric acid. The product recovered in ethyl acetate was purified by flash chromatography, eluted stepwise with the following mixtures of ethyl acetate in hexane containing 0.5% acetic acid: (30, 40, 50, 60, 70 and 85%) (100 cm^3 of each) and 100% (200 cm^3) in 36 fractions. Fractions 24–32 yielded the required ent-3α,10,13-*trihydroxy*-17-*mercaptopropylthio*-20(*norgibberella*-1,16-*diene*-7,19-*dioic acid* 19,10 *lactone* **13** (251 mg) as a gum (Found: M^+, 392.1432. $C_{21}H_{28}O_3S_2$. (*M* − CO_2 − H_2O) requires *M*, 392.1479). $\delta_H[(CD_3)_2CO]$ 1.20 (3 H, s, 18-CH_3), 2.63 (7 H, m, 6-H and 3 × SCH_2), 3.19 (1 H, d, *J* 10, 5-H), 4.04 (1 H, d, *J* 3, H-3), 5.89 (1 H, dd, *J* 9.3, H-2) and 6.36 (1 H, d, *J* 9, H-1); *m/z* 454 (M^+, absent), 393 (87%), 374 (48), 266 (88), 253 (100), 221 (40), 197 (79), 181 (78), 169 (76) and 155 (92).

Reaction of **7** *with Maleic Anhydride*.—Compound **7** (50 mg) in dry dioxane (2 cm^3) was treated with recrystallised maleic anhydride (15 mg) and tributylamine (10 mm^3) for 10 min at room temperature. Ethyl acetate was added, the solvents evaporated and the residue, in ethyl acetate, was eluted through a short column of silica gel with ethyl acetate–light petroleum–

acetic acid (70:30:1). The eluate collected up to a slow moving yellow band, was evaporated and shown by ^{1}H NMR spectroscopy to be the addition product **19**. δ($CDCl_3$) 1.16 (s, 18-CH_3), 2.60 (m, 2 × CH_2S), 2.7–3.12 (complex m, CH_2SCHCO_2, 6-H, CH_2CO_2 and 5-H), 3.98 (br s, 3-H) and 3.99 (dd, *J* 9.5 and 4, $SCHCO_2CO$).

Coupling of [^{3}H]-**7** *or* [^{3}H]-**10** *to Keyhole Limpet Hemocyanin* (*KLH*) *or Bovine Serum Albumin* (*BSA*).—(For **10**) [^{3}H]-**10** (56 mg, 1.4 × 10^6 dpm) in dioxane (1 cm^3) was treated with maleic anhydride (14 mg) and tributylamine (30 mm^3) for 10 min. This solution was added to BSA (60 mg) in borate buffer (pH 9.0) (20 cm^3) and dioxane (10 cm^3) at 4 °C. After the mixture had been stirred overnight at 4 °C, further borate buffer (pH 9.0; 20 cm^3) was added and the solution dialysed against the following solutions: pH 8.5 borate buffer (4 dm^3), pH 8.0 borate buffer (2 dm^3) and water (2 × 2 dm^3), monitoring the release of uncoupled GA_9 by [^{3}H]-radiocounting. When [^{3}H]-GA_9 could no longer be detected in the dialysate, the protein (69 mg) was recovered by lyophilisation. [^{3}H]-Radiocounting of a weighed sample gave 2642 dpm/mg (≡ 105 μgm GA_9-17-S(CH_2)$_3$SH/mg conjugate). Assuming a MW of 68 000 for BSA this gives a coupling ratio (GA_9:BSA) of 17:1.

GA_4–*fluorescein Conjugate* **14**.—Compound **7** (84 mg) in dry tetrahydrofuran (THF) (6 cm^3) with triethylamine (200 mm^3) was cooled in ice under N_2 while a solution of fluorescein isothiocyanate (74 mg) in dry THF (4 cm^3) was added in portions over 1 h. After a further 20 min the solution was added to deoxygenated ethyl acetate–dilute hydrochloric acid. The organic layer was collected and evaporated. The resultant solid was washed with diethyl ether to leave the GA_4–fluorescein conjugate **14** (149 mg) which had λ_{ex}/nm 495 and λ_{em}/nm 520; [+ve ion FAB gave *m*/*z* 830 (M + H$^+$) $C_{43}H_{43}NO_{10}S_3$ requires *M*, 829); δ_H[(CD_3)$_2$CO] 1.1 (18-CH_3), 2.55 (d, *J* 10.5, 6-H), 2.67 (m, 2 × CH_2S), 3.18 (d, *J* 10.5, 5-H), 3.48 (t, *J* 7, CH_2SCS), 3.71 (br d, *J* 2, 3-H), 6.72 (m, 7 × ArH), 7.31 (d, *J* 8, ArH), 8.13 (dd, *J* 8.2, ArH) and 8.60 (br s, NH); δ_C[(CD_3)$_2$CO]. Fluorescyl signals at 198.7 (C=S), 169.0 (C=O), 160.3, 153.3, 150.7, 142.1, 131.1, 130.1, 128.3, 125.2, 113.3, 111.3 and 103.3; GA signals at 178.8 (C_{19}), 174.2 (C_7), 94.5 (C_{10}), 70.3 (C_3), 57.1 (C_4), 55.4 (C_9), 53.7 (C_6), 51.1 and 51.7 (C_5/C_8), 42.8 (C_{15}), 41.5 (C_{16}), 39.1 (C_{13}), 15.96 (C_{11}), 15.2 (C_{18}) and signals not assigned at 35.7, 35.5, 34.6, 31.7, 28.0, 20.9 and two signals masked by solvent (28–31) due to C_1, C_2, C_{12}, C_{14}, C_{17} and $SCH_2CH_2CH_2S$).

GA_4–*dansyl Conjugate* **15**.—Compound **7** (40 mg) in THF (5 cm^3) with *N*-dansylaziridine (30 mg) was stirred while saturated aqueous sodium hydrogen carbonate was added dropwise until the reaction mixture remained cloudy. Stirring was continued for 5 h with occasional further additions of hydrogen carbonate to maintain the opaqueness. The solution was then diluted with water and acidified to pH 3 with hydrochloric acid. The gummy product, recovered in ethyl acetate was purified by flash chromatography in the normal way [30–80% ethyl acetate in light petroleum (0.5% acetic acid)] to give the *dansylated*-GA_4 **15** (39 mg); λ_{ex}/nm 335, λ_{em}/nm 525 (+ve ion FAB gave *m*/*z* 717 (M + H)$^+$; $C_{36}H_{48}N_2O_7S_3$ requires *M*, 716); δ_H[(CD_3)$_2$CO] 1.0 (s, 18-CH_3), 2.48 (d, *J* 10.5, 6-H) superimposed on 2.48 (m, 4 × CH_2S), 2.88 [s, N(CH_3)$_2$], 3.08 (dt, *J* 8 and 6, $CH_2CH_2NHSO_2$), 3.17 (d, *J* 10.5, 5-H), 3.71 (br s, 3-H), 6.88 (t, *J* 6, NH), 7.28 (d, *J* 7.5 ArH), 7.59 (t, *J* 8.5, ArH), 7.63 (dd, *J* 8.5 and 7.5, ArH), 8.24 (d, *J* 7.5, ArH), 8.37 (d, *J* 8.5, ArH) and 8.57 (d, *J* 8.5, ArH).

GA_4–*biotin Conjugate* **10**.—d-Biotin (61 mg) in acetone (10 cm^3) and dimethylformamide (DMF) (1 cm^3) was treated with tributylamine (100 cm^3) and isobutyl chloroformate (35 mm^3). After 1 h compound **7** (100 mg) in acetone (2 cm^3) was added and the reaction left overnight. The solvent was then evaporated and the residue partitioned between ethyl acetate and water containing a few drops of acetic acid. The organic layer was evaporated and the resultant solid was triturated with light petroleum and then dissolved in chloroform, filtered and evaporated to yield GA_4–*biotin* **16** (151 mg) (+ve ion FAB gave *m*/*z* 667 (M + H), $C_{32}H_{46}N_2O_7S_3$ requires *M*, 666); δ_H(CD_3OD) 0.95 (m, biotinyl CH_2), 1.10 (s, 18-CH_3), 2.35 (t, *J* (7, biotinyl-CH_2C), 2.52 (d, *J* 10.5, 6-H), 2.60 (m, 2 × CH_2S), 2.83 (d, *J* 12, biotinyl 1-H), 2.94 (dd, *J* 12, 4.5, biotinyl 1-H), 2.99 (t, *J* 7 Hz, CH_2SC), 3.11 (d, *J* 10.5, 5-H), 3.20 (m, biotinyl-C*H*S), 3.66 (br s, 3-H), 4.30 (dd, *J* 8 and 4.5, biotinyl 1a-H), 4.46 (dd, *J* 8 and 4-H_2, biotinyl 3a-H).

GA_4–*Sepharose Conjugates* **17**.—(*a*) *Activation of Sepharose*. Sepharose 6B (Pharmacia) was washed (×4) with water and sucked dry on a G2 sinter filter until the surface began to crack. This material (40 g) was suspended in water (60 cm^3) and aqueous sodium hydroxide (2 mol dm^{-3}; 26 cm^3) and then epichloroydrin (6 cm^3) were added. The suspension was shaken at 40 °C for 2 h and then sucked dry. It was washed with water (4 × 150 cm^3) and *ca.* 1 g removed to provide a control for microanalysis.

(*b*) *Coupling to* GA_4. The activated Sepharose above (39 g) was washed with phosphate buffer (100 mmol dm^{-3}, pH 8) and then suspended in phosphate buffer–dioxane (2:1) (60 cm^3) containing compound **8** (400 mg). The suspension was shaken overnight at 30 °C and then filtered off. The gel was then washed with phosphate buffer–dioxane (2:1) (2 × 100 cm^3) and then phosphate buffer (100 cm^3).

(*c*) *Blocking of excess epoxy groups*. The gel was then suspended in phosphate buffer (60 cm^3) containing aminoethanol (0.5 mol) for 4 h at 30 °C and then washed with buffer (2 × 100 cm^3) and water (2 × 100 cm^3).

(*d*) *Microanalysis*. The control sample above and a sample of the coupled and blocked gel were freeze-dried and the samples analysed for C, H, N and S. After subtraction of the control values the gel contained 2.58 × 10^{-4} mol sulphur and 3.82 × 10^{-4} mol nitrogen per g. This is equivalent to 126 μmol of gibberellin and 382 μmol of aminoethanol per gram of freeze-dried gel.

17-*Mercaptopropylthio*-GA_4 S-p-*Azidophenacyl Ester* **18**.—To compound **7** (74 mg) in dichloromethane (10 cm^3) was added *p*-azidophenacyl bromide (60 mg) and triethylamine (100 mm^3). After 15 min acetic acid (150 mm^3) was added and the solution evaporated. Flash chromatography (30–70% ethyl acetate in light petroleum containing 0.5% acetic acid) yielded the required *title compound* **18** (79 mg). (Positive ion FAB gave *m*/*z* 600 (M + H)$^+$. $C_{30}H_{37}N_3O_6S_2$ *M*, 599). δ_H[(CD_3)$_2$CO] 1.08 (s, 18-CH_3), 2.59 (m, 6-H and 2 × SCH_2), 3.16 (d, *J* 10.5, 5-H), 3.69 (br s, 3-H), 3.91 (s, SCH_2CO), 7.21 (d, *J* 8.5, 2 × ArH) and 8.07 (d, *J* 8.5, 2 × ArH).

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References

1 M. H. Beale, *J. Chem. Soc., Perkin Trans. 1*, 1990, 925.
2 A. K. Singh, PhD Thesis, University of Bristol, 1988.

3 J. P. Knox, M. H. Beale, G. W. Butcher and J. MacMillan, *Planta*, 1987, **152**, 159.
4 J. E. Nester-Hudson, F. M. Semenko, M. H. Beale and J. MacMillan, *Phytochemistry*, 1990, **29**, 1041.
5 W. H. Scouten, R. Lubcher and W. Baughman, *Biochim. Biophys. Acta*, 1974, **336**, 421.
6 H. D. Knofel, P. Muller, R. Kramell and G. Sembdner, *FEBS Lett.*, 1975, **60**, 39.
7 I. Matsumoto, Y. Mizuno and N. Seno, *J. Biochem.*, 1979, **85**, 1091.
8 I. Matsumoto, N. Seno, A. M. Golovtchenko-Matsumoto and T. Osawa, *J. Biochem.*, 1980, **87**, 535.
9 R. Hooley, M. H. Beale and S. J. Smith, *Planta*, 1991, **183**, 274.
10 R. Hooley, M. H. Beale, S. J. Smith and J. MacMillan, in *Plant Growth Substances*, 1988, eds. R. P. Pharis and S. B. Rood, Springer-Verlag, 1990, p. 145.
11 M. H. Beale, J. MacMillan, C. R. Spray, D. A. Taylor and B. D. Phinney, *J. Chem. Soc., Perkin Trans. 1*, 1984, 541.

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