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Synthesis and bioactivity of labelled germination stimulants for the isolation and identification of the strigolactone receptor †

Anat Reizelman, Suzanne C. M. Wigchert, Cinzia del-Bianco and Binne Zwanenburg*
Department of Organic Chemistry, NSR Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands.
E-mail: zwanenb@sci.kun.nl; Fax: +31-24-3653393; Tel: +31-24-3653159

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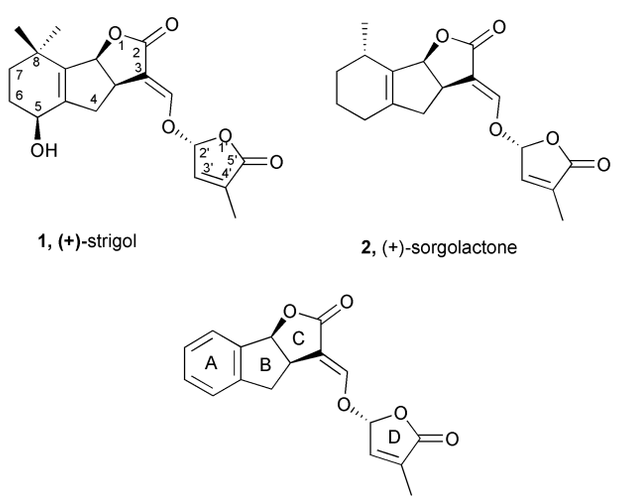
Strigolactones are highly potent germination stimulants for seeds of the parasitic weeds *Striga* and *Orobanche* spp. The induction of seed germination is thought to proceed *via* a receptor-mediated mechanism. Isolation and purification of the strigolactone receptor by affinity chromatography using immobilized avidin or streptavidin requires a biotin labelled strigolactone analogue. For this purpose biotin has been attached, directly as well as indirectly, *via* a hydrophilic linker to the amino function of optically active amino-GR24. Using the same amino substituted synthetic stimulant GR24, labelled stimulants have been prepared which may be suitable for the identification of the receptor by means of fluorescence correlation spectroscopy, scanning force microscopy or photoaffinity techniques. Bioassays of the labelled stimulants reveal that the germination activity on seeds of *Striga hermonthica* is retained. Crystal data for the diastereoisomer (+)-**8** are reported.

Introduction

Parasitic weeds belonging to the genera *Striga* and *Orobanche* severely reduce yields of economically important crops worldwide, especially in tropical and sub-tropical areas.[1] Important food crops, such as maize, sorghum, millet and rice, are host plants that can suffer enormously from these parasitic weeds, leading to considerable losses in crop yield, in some cases more than 50%. Several naturally occurring germination stimulants, *viz*. (+)-strigol[2] **1**, (+)-sorgolactone[3] **2**, (+)-orobanchol[4] and alectrol[5] were isolated from host and non-host plants (Fig. 1). Structure–activity studies of the natural stimulants[6–9] and their synthetic analogues GR7[10] and GR24[11] and Nijmegen-1[12] revealed that: i. Strigolactones are active at a low concentration (10^{-8}–10^{-12}M), ii. The bioactiphore resides in the CD-part of these molecules,[13,14] iii. The absolute configuration at the CD-moiety is of great importance for the seed germination activity.[8,15] These results strongly suggest that the induction of *Striga* and *Orobanche* seed germination proceeds *via* a receptor-mediated mechanism.[8,13,14] So far, nothing is known about the protein structure of this hypothesized receptor, nor of its localization within the seeds. Detailed knowledge of the receptor protein would provide insight in the initial stages of the germination process of these seeds and would enable the design of a perfectly fitting substrate that might be used to control parasitic weed pests by the suicidal germination approach.[16,17,20]



1, (+)-strigol

2, (+)-sorgolactone

3, GR 24

Fig. 1

Affinity chromatography is a widely applied separation technique for the isolation of molecules of biological interest. One popular application involves tagging an antibody or a ligand with biotin and then attaching it to a solid support to which the protein avidin/streptavidin has been linked. Once the antibody or ligand has been attached to the solid support, it can then be used as a stationary phase for the purification of the antigen in the case of an antibody, or of the receptor in case of a ligand. The use of biotin and avidin/streptavidin to link to the solid support depends on the high affinity of avidin or streptavidin for biotin ($K_d = 10^{-14}$–10^{-15} M).[18] To avoid steric hindrance and to allow an optimal association of biotin to streptavidin/avidin usually an extended spacer is incorporated. The ethylene glycol polymers (PEG) have been examined and have the required properties as linkers in biological systems because of their solubility and stability in aqueous solution at physiological pH.

Isolation and purification of the strigolactones receptor by affinity chromatography through immobilized avidin/streptavidin required a biotin labeled strigolactone analogue. A suitable synthetic stimulant for labelling is GR24 **3** (Fig. 1) which exhibits high activity towards *Striga* and *Orobanche* seeds.[19] It should be emphasized that the CD-part is essential for biological activity. The A-ring is in a remote position with respect to the bioactiphore and therefore, various substituents have been introduced in this ring without seriously affecting the bioactivity.[20] This then leads to the strategy outlined in Fig. 2 for the synthesis of appropriately labelled stimulants on the basis of GR24.

In fact, the synthesis of some biologically active, labelled strigolactone analogues suitable, at least in principle, for the identification of the strigolactone receptor, has been reported previously from our laboratory.[20] In order to achieve optimal binding to the receptor, the stereochemistry of the stimulant moiety should be the same as that of natural strigolactones. In this paper, the synthesis of optically active amino-GR 24 and

† Electronic supplementary information (ESI) available: Nitration of (+)-**4**, preparation of (+)-**5**, *rac*-**7** and *rac*-**8**, details of the X-ray analysis of (+)-**8**. See http://www.rsc.org/suppdata/ob/b2/b210678g/

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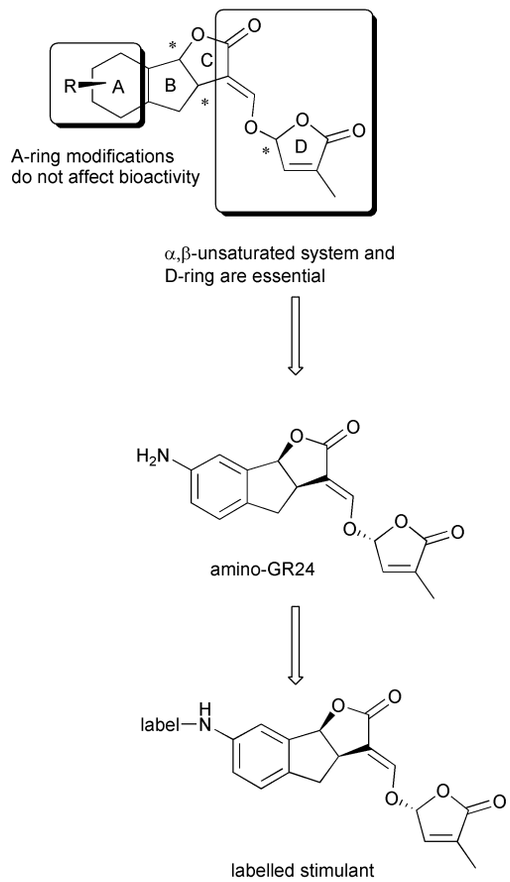


Fig. 2

the subsequent attachment of biotin directly as well as *via* a hydrophilic spacer (PEG) is described.

Several other methods can be considered to gather information about the protein receptor, *e.g.* fluorescence correlation spectroscopy (FCS),[21,22] scanning force microscopy (SFM)[23] and photoaffinity labelling (PAL).[24,25,26] In FCS the labelled substrate is exposed to a focused laser beam whereby fluorescent molecules will be excited to give a burst of photons that can be detected. FCS is attractive for the current problem as it is highly sensitive, provided efficient fluorescent labels are used. In this paper, the preparation of a strigolactone analogue with such a fluorescent tag, using the strategy shown in Fig. 2, is described. In SFM the ligand, which responds to a receptor, is attached to a gold SFM-tip using a linker with a free SH group. A strigolactone analogue containing such a SFM linker has been prepared. PAL requires the attachment of a photoreactive unit to the ligand, *e.g.* an azido group, allowing the generation of a very reactive, short-lived intermediate upon irradiation, which will immediately form a covalent bond with the molecule in its nearest vicinity, *i.e.* the protein receptor. A strigolactone analogue containing a photoreactive tag has been prepared from amino-GR24. In all strigolactone analogues mentioned above a suitable substituent has been introduced at the A-ring of GR24. In order to serve their purpose in the isolation or identification of the strigolactone receptor these analogues must have germination activity. Thus, bioassays of these compounds with seeds of *Striga hermonthica* have been performed.

Results and discussion

Synthesis

Enantiopure amino-GR24 (+)-**7**, having the same configuration as the natural strigolactones, is a key compound in the synthesis of labelled strigolactone analogues. The synthesis involves the coupling of chlorobutenolide[27] *rac*-**6** to enantiopure amino-ABC-lactone (−)-**5** as depicted in Scheme 1. In order to obtain the desired optically pure (−)-**5**, racemic tricyclic lactone *rac*-**4** was chromatographically resolved using cellulose triacetate, as described previously.[19b] Nitration of (−)-**4** followed by reduction of the nitro group to an amine substituent was performed using tin and hydrochloric acid according to the method of Thuring *et al.*[20] The nitration also gave some C_5-nitro product (ratio C_7-NO_2 : C_5-NO_2 = 9 : 1). The preference for nitration at C_7 can be explained by the higher electron density at that carbon atom. Prior to formylation and coupling with chlorobutenolide *rac*-**6**, the amino substituent was protected with a *tert*-butyloxycarbonyl (Boc) group. Formylation of (−)-**5** employing sodium in ethyl formate as the solvent, followed by reaction of the resulting sodium enolate with *rac*-**6** in THF gave protected enantiopure amino-GR24 diastereoisomers (+)-**7** and (+)-**8**. These two diastereoisomers could readily be separated by chromatography on silica gel. In a similar manner, enantiopure amino-GR24 diastereomers (−)-**7** and (−)-**8** were synthesized from tricyclic lactone (+)-**5**. The stereochemistry of these four diastereoisomers of amino-GR24 was secured as follows. The absolute stereochemistry of the tricyclic lactones (−)-**4** and (−)-**5** is correlated with GR24 having the natural configuration at C_{8b} and C_{3a}, namely *S* and *R*, respectively.[19b] CD spectra can now be used to determine the stereochemistry at C2′in (+)-**7** and (−)-**7**. It has been demonstrated by Welzel *et al.*,[28,29] that the sign of the Cotton effect at 270 nm is directly correlated with the stereochemistry at C2′, namely, a negative Cotton effect at this wavelength corresponds with the C2′(*R*) configuration and a positive sign with the C2′(*S*) configuration. This rule has proven its value in establishing the stereochemistry in several strigolactones, especially GR28,[30] desmethyl sorgolactone,[15] sorgolactone[8] and strigol.[9] Based on CD spectra the absolute configuration at C2′ of (+)-**7** is *R* and of (−)-**7** is *S*. This assignment is in accordance with the germination stimulatory activity towards seeds of *Striga hermonthica*. The diastereoisomer (+)-**7** having the natural configuration at all its stereogenic centres shows an appreciable activity at a concentration of 10^{-8} M, whereas its antipode (−)-**7** hardly exhibits any activity at the same concentration (see Table 1). In addition, an X-ray analysis was performed on the slow moving diastereoisomer derived from tricyclic lactone (−)-**5**. A PLUTON generated drawing of the crystal structure of (+)-**8** is shown in Fig. 3. It should be noted that the refinement process of this X-ray analysis needed special care to obtain consistent values for the average displacement parameters, distances and bond angles. This diastereoisomer (+)-**8** has *S* configuration at C2′, which stereochemistry at the B–C ring junction is the same as in (+)-**7**.

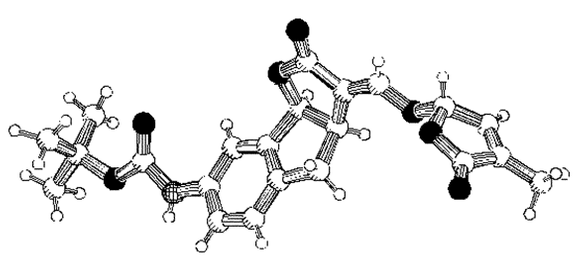
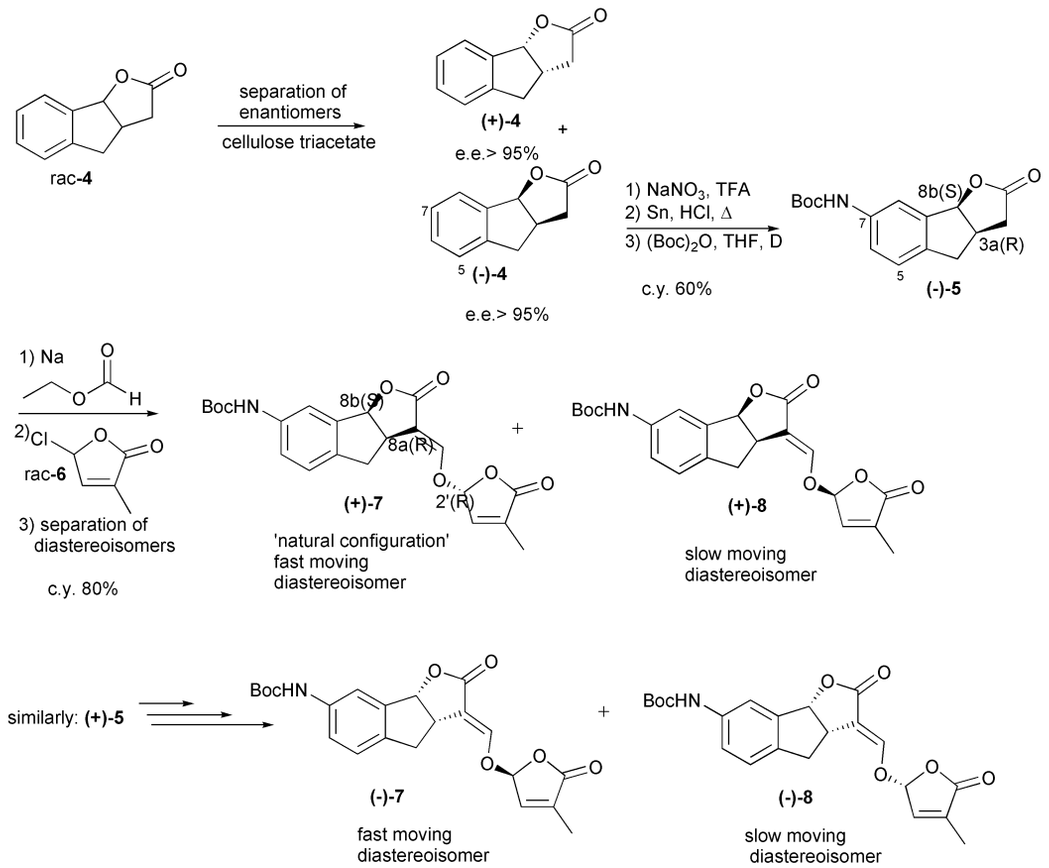


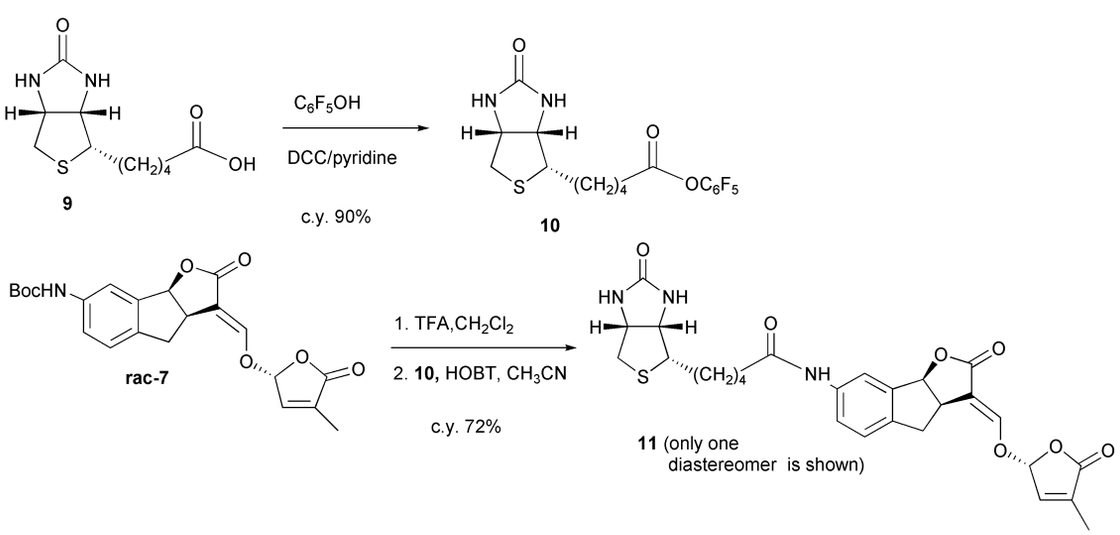
Fig. 3 X-Ray structure of (+)-**8**.

The coupling of diastereoisomer *rac*-**7** with biotin was investigated next. Removal of the Boc group, followed by coupling with biotin **9** using DCC–DMAP did not meet with success. Therefore, an activated ester, namely the pentafluorophenyl (Pfp) ester[31] **10** was considered. Gratifyingly, a smooth coupling with amino-GR24 took place in the presence of HOBT, to give the product **11** as an inseparable mixture of diastereoisomers in 72% yield (Scheme 2).

Then attempts were made to incorporate a linking spacer **12**. This linker **12** was chosen in order to improve the water



Scheme 1 Preparation of all four amino-GR24 stereoisomers.

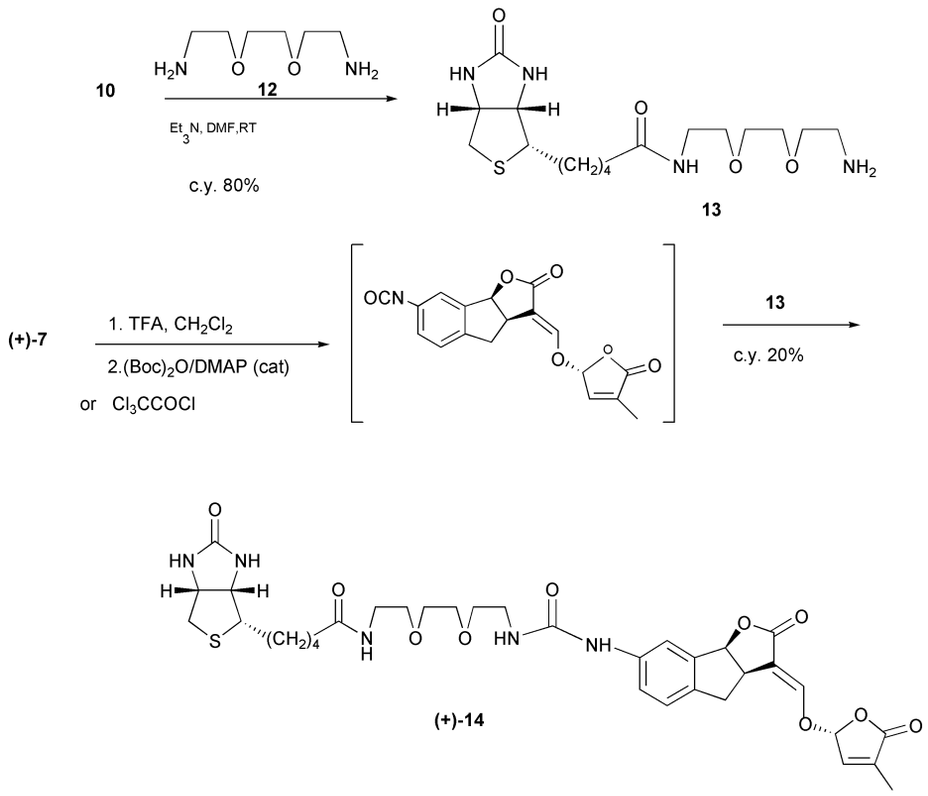


Scheme 2 Synthesis of biotin labeled GR24 (**11**).

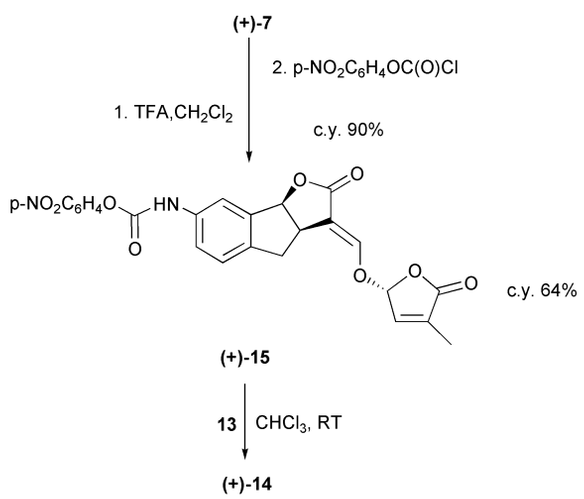
solubility of the biotin derivative. The coupling of biotin Pfp ester with bisamine **12** gave amide **13** following a slightly modified literature procedure.[32] Coupling of **13** with amino-GR24 was accomplished *via* the isocyanate derived from amino-GR24 upon reaction with $(Boc)_2O$ or trichloroacetyl chloride. Starting from diastereoisomer (+)-**7** biotin labelled compound (+)-**14** was obtained in a modest yield of 20% (Scheme 3). Conversion of the biotin substrate **13** into the corresponding isocyanate and its subsequent *in situ* coupling with (+)-**7** gave unsatisfactory results. However, the alternative coupling of *p*-nitrophenyl carbamate (+)-**15**, obtained from (+)-**7** by reaction with *p*-nitrophenyl chloroformate, with **13** resulted in the desired biotin labelled GR24 analogue in a good yield (64%) (Scheme 4).

In order to exclude non-specific binding during the receptor protein identification procedure, a negative control must be included. Biotin labeled ABC-lactone **17** is a suitable candidate for this purpose as it is lacking the vinyl enol ether moiety and the D-ring, which are proposed as the bioactiphore for the strigolactone receptor[14] (Scheme 5). Compound **17** was prepared in the same manner as described for (+)-**14** again using a *p*-nitrophenyl carbamate, *viz*. **16**. *Rac*-**5** gave an inseparable mixture of diastereoisomers of **17** in 89% yield (Scheme 5).

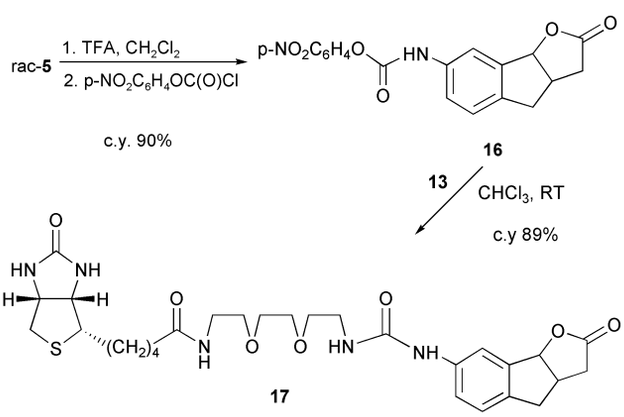
A labelled strigolactone analogue suitable for FCS studies requires a fluorescent tag with an absorption maximum at the wavelength of the FCS-laser. Tetramethylrhodamine (λ_{max}Abs = 543 nm) is suited for excitation at the 543 nm spectral line of the green Helium-Neon laser.[33] The reactive isothiocyanate of tetramethylrhodamine (TRIC) was chosen for coupling to



Scheme 3 Incorporating a PEG spacer into biotin labelled GR24.



Scheme 4 Synthesis of biotin labelled amino-GR24.

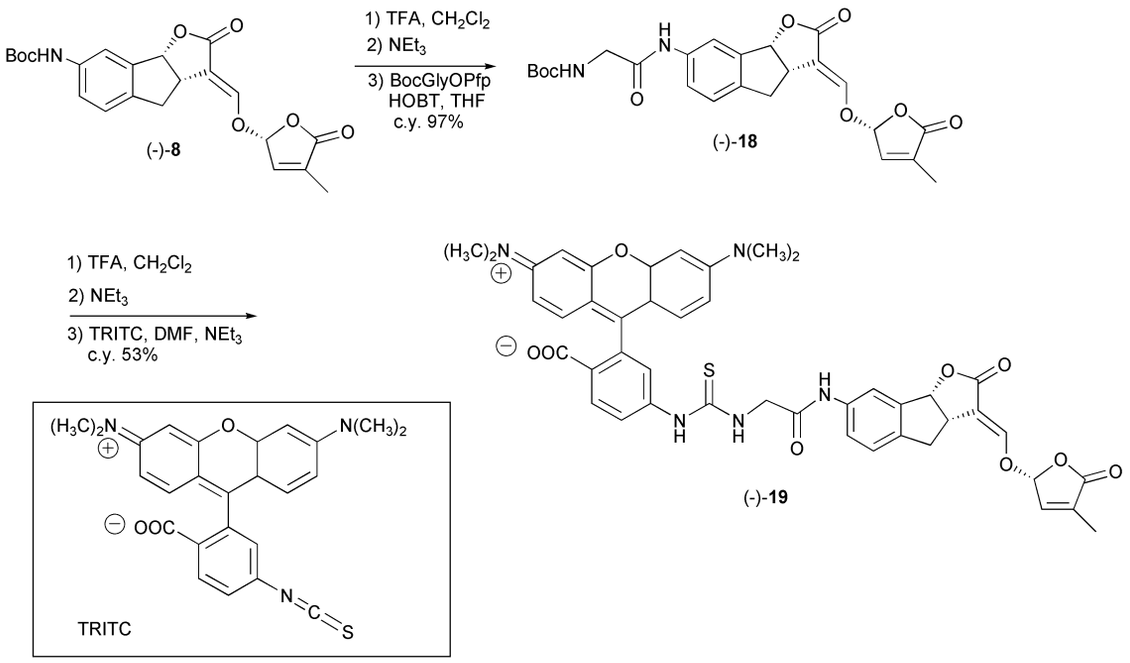


Scheme 5 Preparation of biotin labelled amino-GR 24 ABC-part.

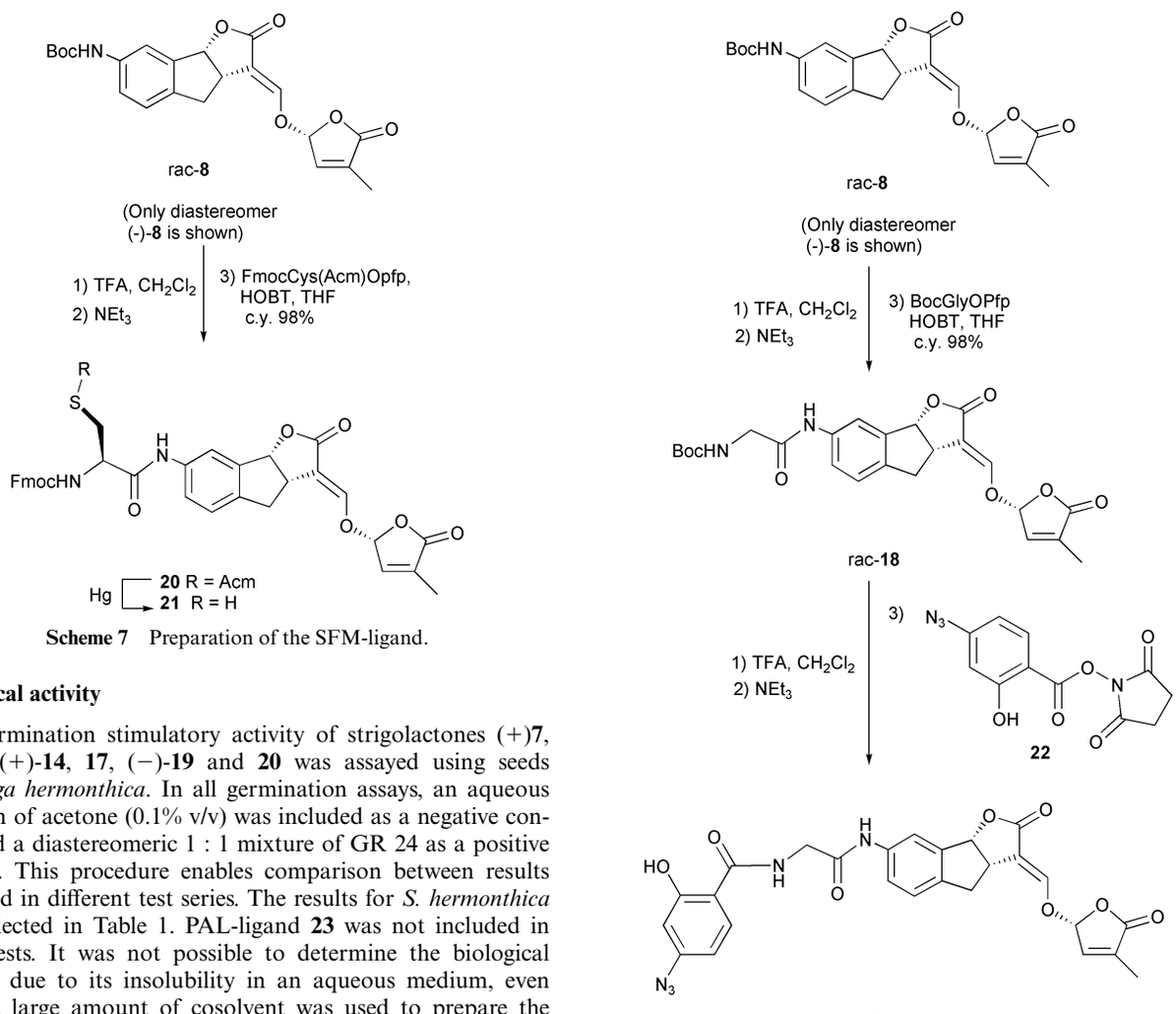
amino-GR24 derived from (−)-**8**. However, the aromatic amine group was not very reactive towards TRIC. Therefore, a glycine spacer was linked to the aromatic amine, which was readily performed *via* coupling with the PfP ester of glycine in the presence of HOBT (Scheme 6). Removal of the Boc protective group from (−)-**18**, gave a highly reactive primary amine, which smoothly reacted with TRIC. Isolation of the intermediate primary amine was not possible, because of its rapid conversion to the corresponding carbamic acid by reaction with CO_2 in air. Fluorescently labelled strigolactone analogue (−)-**19** was purified by silica gel chromatography and isolated as a bright pink powder in 53% yield.

A ligand that can be used as a probe in SFM, requires the presence of an SH group. Since coupling of the amine derived from *rac*-**8** with an amino acid PfP ester proceeded smoothly, this reaction was also chosen for the introduction of the SH moiety into the GR24 derivative. Commercially available cysteine derivative FmocCys(Acm)OPfP was reacted *in situ* with the aromatic amine to give compound **20** (Scheme 7). The acetamido methyl (Acm) protective group can be removed under natural conditions in the presence of mercury.[34] In principle, ligand **21** can be linked to a gold SFM tip. The position of the SH group in the ligand is such that steric interference with the bioactiphore of the strigolactone analogue is unlikely. It is not strictly necessary to remove the Fmoc group, provided the molecule is sufficiently active in the germination of parasitic weed seeds. The synthetic scheme for **21** can also be used for enantiomerically pure material, which then can be employed to coat the gold probe for the SFM-studies.

PAL needs a ligand containing a photoreactive group. It is highly desirable to include this group in the final synthetic step, to minimize the risk of premature activation of the light sensitive unit. To demonstrate the feasibility of the synthetic strategy, succinimidyl ester **22** was reacted with strigolactone analogue *rac*-**18** after deprotection (Scheme 8). The coupling with the aliphatic primary amine of the spacer proceeded in quantitative yield, whereas the aromatic amino-GR24 was much less reactive.



Scheme 6 Preparation of the FCS ligand.



Scheme 8 Preparation of the PAL-ligand.

Biological activity

The germination stimulatory activity of strigolactones (+)**7**, (−)-**7**, (+)-**14**, **17**, (−)-**19** and **20** was assayed using seeds of *Striga hermonthica*. In all germination assays, an aqueous solution of acetone (0.1% v/v) was included as a negative control and a diastereomeric 1 : 1 mixture of GR 24 as a positive control. This procedure enables comparison between results obtained in different test series. The results for *S. hermonthica* are collected in Table 1. PAL-ligand **23** was not included in these tests. It was not possible to determine the biological activity due to its insolubility in an aqueous medium, even when a large amount of cosolvent was used to prepare the stock solution of this compound. For *S. hermonthica*, labelled compounds (+)-**14**, **19** and **20** stimulated the germination of the weed seeds, confirming that a large A-ring substituent is indeed tolerated by the strigolactone binding site of *S. hermonthica* seeds. As expected compound **17** exhibits no activity in these seeds. It should be noted however, that compound **20** was also rather insoluble in aqueous medium. The germination values reported for this compound were difficult to reproduce, probably due to crystalisation of the stimulant in the aqueous solution. In all assays, the aqueous blanks had not induced germination of the seeds.

Table 1 Percentages of germinated seeds of *S. hermonthica* after exposure to solutions (2×10^{-6} and 2×10^{-8} mol L^{-1}) of (+)-**7**, (−)-**7**, (+)-**14**, **17**, (-)-**19**, **20** relative to the control GR24 (**3**)[a]

Entry	Compound	% germination ± SE at a concentration of 2×10^{-6} mol L^{-1}	% germination ± SE at a concentration of 2×10^{-8} mol L^{-1}
1	(+)-**7**	78 ± 5.1	90 ± 6.2
2	(-)-**7**	68 ± 4.5	10 ± 1.2
3	(+)-**14**	61 ± 2	70 ± 5
4	**17**	4 ± 1.1[c]	3 ± 1.1[c]
5	(-)-**19**	130 ± 4.9	66 ± 0.7
6	**20**	74 ± 8.1	37 ± 3.6
7[d]	**GR24**[b]	100	100

[a] Data presented the mean ± SE of one representative experiment and are relative to GR24. [b] Equimolar mixture of two racemic diastereomers. [c] Not significantly different from aqueous control (without stimulant). [d] The average germination of GR24 was found to be in a range of 50–60%.

Fluorescent stimulant (−)-**19** dissolved well in water because of its ionic nature.

Interestingly, compounds (−)-**19** and **20** which were prepared from the diastereoisomer (−)-**8**, were found to induce germination of the seeds of *S. hermonthica*. Diastereoisomer (−)-**8** possesses the same stereochemistry as the natural strigol at C′2, but opposite stereochemistry at C8b and C3a. However, it was demonstrated earlier that the stereochemistry at C2′ is essential for biological activity, whereas the stereochemistry at C8b and C3a has a minor effect on the germination.[15] Furthermore, the lack of a significant reduction in the stimulation activity found with these compounds (see Table 1) suggests that they would be suitable candidates for isolation of the strigolactone receptor.

In summary, we have prepared labelled strigolactone analogues tagged with a fluorescent, radioactive, photoaffinity moiety and biotin group for the isolation and identification of the strigolactone receptor. Amino-GR24 is the appropriate starting material for the attachment of various tags to the A-ring. Bioassays of the labelled stimulants reveal that the germination activity on seeds of *S. hermonthica* is predominantly retained. Preliminary results in the isolation of strigolactone receptor, using the synthetic biotinylated strigolactone analogues, showed a binding protein present in the membrane fractions of *Striga hermonthica* seeds. These results will be reported elsewhere in due time.[35]

Experimental

General remarks

^{1}H NMR (300 MHz) and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer, using Me_4Si as internal standard. All coupling constants are given as ^3J in Hz, unless indicated otherwise. Melting points were measured with a Reichert thermopan microscope and are uncorrected. IR spectra were recorded on a Bio-Rad FTS-25 instrument. For mass spectra a double focusing VG7070E mass spectrometer was used. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separations were carried out on a fused-silica capillary column (DB-5, 30 m × 0.25 mm), helium was used as the carrier gas and electron impact (EI) was used as ionization mode. Elemental analyses were conducted on a Carlo Erba Instrument CHNSO EA 1108 element analyser. For the determination of optical rotations a Perkin-Elmer 241 polarimeter was used. Optical rotations are given in 10^{-1} deg cm^2 g^{-1}. Solvents were dried using the following methods: dichloromethane was distilled from P_2O_5; ethyl acetate was distilled from K_2CO_3; diethyl ether was distilled from NaH; hexane was distilled from CaH_2; tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade and used as purchased. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm). Spots were visualized with UV or using molybdate spray. Flash chromatography was carried out at a pressure of *ca*. 1.5 bar, using Merck Kiesegel 60H. Column chromatography at atmospheric pressure was performed with Merck Kieselgel 60. Enantiomeric excesses of compounds **7** and **8** were determined by analytical HPLC using Chiralcel OD (10 μm) cellulose carbamate column (Baker, 250 × 4.6 mm) using mixtures of 2-propanol or ethanol and hexane as the eluent. CD-spectra were recorded using a Jasco spectrophotometer.

Synthesis

7-Nitro-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one

Tricyclic lactone (−)-**4** (2.9 g, 16.7 mmol) was nitrated as described by Thuring *et al*.[20] Recrystallization from toluene afforded pale yellow needles. Yield: 74%. Mp: 118–119 °C. $[\alpha]_D^{22}$ −197 (c = 0.4, CH_2Cl_2). All other analytical data were identical with those reported previously for the racemic compound.[20]

tert-Butyl *N*-[2-oxo-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno-[1,2-*b*]furan-7-yl]carbamate (−)-5

Reduction of nitro tricyclic lactone derived from (−)-**4** (2.66 g, 12.15 mmol) was accomplished in quantitative yield with tin and hydrochloric acid as described previously for the racemic compound.[20] A mixture of 7-amino-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (2.0 g, 10.6 mmol) and di-*tert*-butyl dicarbonate (3.5 g, 16 mmol) was heated under reflux in THF (25 mL). After 2.5 h, the mixture was cooled and THF was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed with tartaric acid (1 M, 1×), dried ($MgSO_4$) and concentrated. Silica gel chromatography (hexane–ethyl acetate 1 : 1) yielded (−)-**5** (2.6 g, 85%) as a white solid. Pure (−)-**5** was obtained by crystallization from toluene following by second recrystallization from hexane–ethyl acetate mixture afforded colourless needles. Mp: 135–136 °C. $[\alpha]_D^{22}$ −158.5 (c = 0.4, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (s, 9H, 3 × C*H*$_3$ tBu), 2.37 (dd, 1H, J_1 = 5.8 Hz, J_2 = 18.0 Hz, *H*3), 2.82 (dd, 1H, J_1 = 3.4 Hz, J_2 = 16.3 Hz, *H*4), 2.88 (dd, 1H, J_1 = 9.8 Hz, J_2 = 18.0 Hz, *H*3), 3.25 (dd, 1H, J_1 = 8.3 Hz, J_2 = 16.3 Hz, *H*4), 3.37 (m, 1H, *H*3a), 5.83 (d, 1H, J = 7.2 Hz, *H*8b), 6.54 (br s, 1H, N*H*), 7.17 (d, 1H, J = 8.2 Hz, *H*5), 7.33 (d, 1H, J = 8.3 Hz, *H*6), 7.51 (s, 1H, *H*8). ^{13}C NMR ($CDCl_3$): δ 28.2 (3C, *C*H$_3$ tBu), 35.6, 37.3 (2C, *C*H$_2$), 37.7 (1C, *C*H3a), 80.7 (1C, *C*q tBu), 87.5 (1C, *C*H8b), 116.3, 120.8, 125.6 (3C, *C*H$_{arom}$), 136.8, 138.0, 139.6 (3C, *Cq*$_{arom}$), 141.7 (1C, *C*H3′), 152.7, 176.8 (2C, CO). IR (KBr): ν/cm^{-1} 3338, 1756, 1726, 1180. MS [EI *m*/*z*, rel. intensity (%)]: 289 ([M]$^+$, 23.8), 233([$C_{12}H_{11}O_4N$]$^+$, 14.5), 189([$C_{11}H_{11}O_2N$]$^+$, 24.0), 57([C_4H_9]$^+$, 81.3), 28([CO]$^+$, 100). Anal. calcd. for $C_{16}H_{19}O_4N$: C, 66.42; H, 6.62; N, 4.84 found: C, 66.20; H, 6.60; N, 4.87%.

tert-Butyl *N*-[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*R*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]carbamate (−)-7 and its 2(*S*) diastereoisomer: *tert*-butyl *N*-[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*S*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]carbamate (−)-8

To a cooled (0 °C) and stirred solution of Boc-amino tricyclic lactone (+)-**5** (650 mg, 2.25 mmol) in ethyl formate (10 ml) was added, under continuous stream of nitrogen, 2.2 equiv. of metallic sodium (111 mg, 4.8 mmol). The mixture was allowed to warm to room temperature and stirred for 1.5 hour. When

TLC analysis indicated complete formylation excess ethyl formate was removed by evaporation *in vacuo*. The thus obtained sodium salt of formylated (+)-**5** was suspended in THF (10 mL) and cooled to 0 °C. Upon addition of chlorobutenolide **6** (583 mg, 4.4 mmol) the reaction mixture became clear. The mixture was stirred overnight. Then THF was removed *in vacuo* and the residue was dissolved in a mixture of brine and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2 × 20 mL) and combined organic layers were washed with saturated NH_4Cl (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified using flash chromatography (SiO_2, hexane–ethyl acetate 2 : 1) to afford two diastereoisomeric products. Fast moving diastereoisomer (−)-(**7**) (400 mg, 44%) and slow moving diastereoisomer (−)-**8** (380 mg, 42%) were obtained as white solids after recrystallization from hexane–ethyl acetate.

(−)-**7**: mp: 115–117 °C. Ee >99% (determined by HPLC). $[a]_D^{22}$= −331.6 (c = 0.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51 (s, 9H, 3 × CH_3 tBu), 2.04 (s, 3H, CH_3 D-ring), 3.04 (dd, 1H, J_1 = 3.2 Hz, J_2 = 16.7 Hz, *H*4), 3.37 (dd, 1H, J_1 = 9.3 Hz, J_2 = 16.7 Hz, *H*4), 3.94 (m, 1H, *H*3a), 5.90 (d, 1H, J = 7.9 Hz, *H*8b), 6.17 (m, 1H, *H*2′), 6.50 (br s, 1H, N*H*), 6.96 (m, 1H, *H*3′), 7.15 (d, 1H, J = 8.3 Hz, *H*5), 7.35 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.7 Hz, *H*6), 7.47 (d, 1H, J = 2.5 Hz, *H*6′), 7.50 (d, 1H, J = 1.7 Hz, *H*8). ^{13}C NMR ($CDCl_3$): δ 11.3 (*C*H$_3$ D-ring), 28.9 (3C, *C*H$_3$ tBu), 37.3 (1C, *C*H$_2$), 39.9 (1C, *C*H3a), 81.1 (1C, *Cq* tBu), 86.4 (1C, *C*H8b), 101.3 (1C, *C*H2′), 113.7 (1C, *Cq* C-ring), 116.9, 121.6, 126.0 (3C, *C*H$_{arom}$), 136.3, 137.5, 138.6, 140.2 (3C, Cq$_{arom}$ and 1C, Cq D-ring), 141.7 (1C, CH3′), 151.7 (1C, CH6′), 153.4, 170.9, 171.8 (3C, CO). MS [CI *m*/*z* rel. intensity (%)]: 413 ([M]$^+$, 12.5); 357 ([$C_{18}H_{15}O_7N$]$^+$, 88.8); 313 ([$C_{17}H_{15}O_5N$]$^+$, 9.3); 217 ([$C_{12}H_{11}O_3N$]$^+$, 21.4); 97 ([$C_5H_5O_2$]$^+$, 32.2), 57 ([C_4H_9]$^+$, 100). Anal. calcd. for $C_{22}H_{23}O_7N$: C, 63.92; H, 5.61; N, 3.39 found: C, 64.06; H, 5.73; N, 3.31%.

(−)-**8**: mp: 226–228 °C (decomp.). Ee >99% (determined by HPLC). $[a]_D^{22}$= −323.6 (c = 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.53 (s, 9H, 3 × CH_3 tBu), 2.04 (s, 3H, CH_3 D-ring), 3.03 (dd, 1H, J_1 = 3.1 Hz, J_2 = 16.7 Hz, H4), 3.35 (dd, 1H, J_1 = 9.2 Hz, J_2 = 16.7 Hz, *H*4), 3.93 (m, 1H, *H*3a), 5.90 (d, 1H, J = 7.8 Hz, *H*8b), 6.16 (m, 1H, *H*2′), 6.48 (br s, 1H, N*H*), 6.96 (m, 1H, *H*3′), 7.13 (d, 1H, J = 8.3 Hz, *H*5), 7.30 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.8 Hz, *H*6), 7.46 (d, 1H, J = 2.5 Hz, *H*6′), 7.53 (d, 1H, J = 1.8 Hz, *H*8). ^{13}C NMR ($CDCl_3$): δ 11.4 (1C, *C*H$_3$ D-ring), 29.0 (3C, *C*H$_3$ tBu), 37.5 (1C, *C*H$_2$), 39.9 (1C, *C*H3a), 81.3 (1C, *Cq* tBu), 86.5 (1C, *C*H8b), 101.3 (1C, *C*H2′), 114.0 (1C, *Cq* C-ring), 116.9, 121.7, 126.2 (3C, *C*H$_{arom}$), 136.6, 138.5, 138.6, 140.3 (3C, *Cq*$_{arom}$ and *Cq* D-ring), 141.7 (1C, *C*H3′), 151.6 (1C, *C*H6′), 153.4, 170.9, 171.9 (3C, *C*O). MS [CI *m*/*z* rel. intensity (%)]: 413 ([M]$^+$, 15.8); 357 ([$C_{18}H_{15}O_7N$]$^+$, 44.7); 313 ([$C_{17}H_{15}O_5N$]$^+$, 25.0); 217 ([$C_{12}H_{11}O_3N$]$^+$, 73.4); 97 ([$C_5H_5O_2$]$^+$, 68.2), 57 ([C_4H_9]$^+$,100). Anal. calcd. for $C_{22}H_{23}O_7N$: C, 63.92; H, 5.61; N, 3.39 found: C, 64.06; H, 5.70; N, 3.21%.

tert-Butyl *N*-[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*S*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*S*),4,8b(*R*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]carbamate (+)-7 and its 2(*R*) diastereoisomer: *tert*-butyl *N*-[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*R*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*S*),4,8b(*R*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]carbamate (+)-8

Boc-amino-GR24 diastereoisomers (+)-**7** and (+)-**8** were prepared in the same way as described for their enantiomers (−)-**7** and (−)-**8**, starting from carbamate (−)-**5** (770 mg, 2.7 mmol).Yield (+)-**7**: 43%; (+)-**8**: 38%. Analytical samples were obtained by recrystallization from 2-methyl-2-butanol.

(+)-**7**: mp: 116–118 °C. Ee >99% (determined by HPLC). $[a]_D^{22}$ = +338.7 (c = 0.1, CH_2Cl_2). Anal. calcd. for $C_{22}H_{23}O_7N$: C, 63.92; H, 5.61; N, 3.39 found: C, 64.10; H, 5.73; N, 3.13%. All other analytical data were the same as reported for (−)-**7**.

(+)-**8**: mp: 228–230 °C. Ee >99% (determined by HPLC). $[a]_D^{22}$ = +331.5 (c = 0.1, CH_2Cl_2). Anal. calcd. for $C_{22}H_{23}O_7N$: C, 63.92; H, 5.61; N, 3.39 found: C, 63.63; H, 5.62; N, 3.44%. All other analytical data were the same as reported for (−)-**8**.

*N*1-[(3a*R*,8b*S*)-3-((*E*)-1-[(2*R*)-4-Methyl-5-oxo-2,5-dihydro-2-furanyl]oxymethylidene)-2-oxo-3,3a,4,8b-tetrahydro-2*H*-indeno-[1,2-*b*]furan-7-yl]-5-(2-oxoperhydrothieno[3,4-*d*]imidazol-4-yl)pentanamide 11

Complete deprotection of *rac*-**7** (20 mg, 0.064 mmol), obtained in the same manner as (−)-**7**, starting from *rac*-**5**, was carried out using 1.5 mL TFA and 10 mL CH_2Cl_2 after 1 h. After evaporation to remove the excess acid, EtOAc was added and pH 8 was adjusted by addition of saturated $NaHCO_3$. The organic layer was then washed with a little cold water and dried ($MgSO_4$). Volatiles were evaporated and the free amine was dissolved in dry acetonitrile (20 mL). Then **10**[31] (28 mg, 0.068 mmol) and hydroxybenzotriazole (HOBT, 10 mg, 0.07 mmol) were added and the mixture was stirred at room temperature for 12 h. Acetonitrile was removed *in vacuo* and the residue was purified by column chromatography (SiO_2; CH_3OH–CH_2Cl_2 7 : 1) to give **11** as a white solid (20 mg, 72% yield). TLC: ($CHCl_3$–MeOH 5 : 1, R_f = 0.31). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (m, 6H, biotin-CH_2), 2.03 (s, 3H, CH_3 D-ring), 2.33 (t, 2H, J = 7.5 Hz, CH_2CO), 2.70 (d, 1H, J = 12.6 Hz, CHH_{endo}S), 2.84 (dd, 1H, J_1 = 12.6 Hz, J_2 = 4.5 Hz, 1H, CHH_{exo}S), 3.08 (m, 2H, C*H*S and *H*4), 3.33 (dd, 1H, J_1 = 16.7 Hz, J_2 = 9.3 Hz, *H*4), 3.91 (m, 1H, *H*3a), 4.31, 4.49 (m, 2H, C*H*NH), 5.32, 5.51 (2s, 1H, N*H*), 5.88 (d, J_1 = 7.5 Hz, 1H, *H*8b), 5.97 (br t, 1H, N*H*), 6.21 (s, 1H, *H*2′), 6.33, 6.79 (s, 2H, N*H*), 6.99 (m, 1H, *H*3′), 7.11 (d, 1H, J = 8.4 Hz, *H*5), 7.48 (m, 1H, *H*8), 7.59 (d, 1H, J = 9.0 Hz, *H*6), 7.69 (m, 1H, *H*6), 8.86 (s, 1H, N*H*).^{13}C NMR ($CDCl_3$): δ 174.18, 171.8, 170.9, 163.97, 156.25 (5C, *C*O), 151.58 (1C, *C*H6′), 141.1 (1C, *C*H3′), 139.64, 139.28, 135.77, 135.69 (4C, 3*Cq*$_{arom}$ and Cq D-ring), 125.37, 121.38, 116.16 (3C, *C*H$_{arom}$), 112.96 (1C, *Cq*C-ring), 100.73 (1C, *C*H2′), 86.15 (1C, *C*H8b), 61.79, 60.13 (2C, *C*HNH), 55.41 (1C, *C*HS), 40.44 (1C, *C*H$_2$S), 39.72 (1C, *C*H3a), 39.41, 39.29 (2C, *C*H$_2$NH), 36.55 (1C, *C*H$_2$), 35.98 (1C, *C*H$_2$CO), 28.05, 27.91, 25.48 (3C, *C*H$_2$), 22.85 (1C, *C*H$_3$ D-ring); MS [FAB *m*/*z*, rel. intensity (%)]), 539 ([M]$^+$, 100). Anal. calcd. for $C_{27}H_{29}N_3O_7S$: C, 60.10; H, 5.42; N, 7.79 found: C, 60.36; H, 5.73; N, 7.90%.

*N*1-2-[2-(2-Aminoethoxy)ethoxy]ethyl-5-(2-oxoperhydrothieno[3,4-*d*]imidazol-4-yl)pentanamide 13

A solution of **10**[31] (0.5 g, 1.21 mmol) in anhydrous DMF (25 mL) was added dropwise over 1 hour to a mixture of aminoethoxyethoxyethylamine **12** (2.0 mL, 12 mmol), and Et_3N (0.5 mL, 3.6 mmol). The reaction was stirred at room temperature for 30 min and the solution was concentrated *in vacuo*. The resulting oil was triturated in 50 mL ether and filtered. The crude mixture was purified by a counter current extraction (butanol–water 1 : 1) and freeze-dried. TLC control (butanol–water–acetic acid 4 : 1 : 1) showed almost no bis-substituted diamine. The thus obtained white solid (0.4 g, 87% yield with respect to biotin) was used without further purification. TLC (butanol–water–acetic acid 4 : 1 : 1 R_f = 0.25). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 1.4 (m, 6H, biotin-CH_2), 1.6 (s, 2H, NH_2), 2.06 (t, 2H, J = 7.5 Hz, CH_2CO), 2.52 (d, 1H, J = 12.45 Hz, CHH_{endo}S), 2.64 (t, 2H, J = 6 Hz, CH_2–NH$_2$), 2.81 (dd, 1H, J_1 = 12.0 Hz, J_2 = 4.8 Hz, 1H, CHH_{exo}S), 3.10 (m, 1H, C*H*S), 3.16 (m, 2H, CH_2NH), 3.3 (m, 4H, OCH_2), 3.5 (s, 4H, OCH_2CH_2O), 4.29, 4.11 (m, 2H, C*H*NH), 6.42, 6.35 (s, 2H, N*H*), 7.85 (t, 1H, J = 5.7 Hz, N*H*CO). ^{13}C NMR (DMSO-d$_6$): δ 172.12, 162.70 (2C, *C*O), 72.80, 69.55, 69.51, 69.15 (4C, *C*H$_2$O), 61.02, 59.17 (2C, *C*HNH), 55.41 (1C, *C*HS), 41.21 (1C, *C*H$_2$S), 38.44 (2C, *C*H$_2$NH), 35.08 (1C, *C*H$_2$CO), 28.18, 28.03, 25.26 (3C, *C*H$_2$); IR (KBr): ν/cm^{-1} 3289, 3081, 2927, 2862, 1701, 1645, 1553, 1461, 1423, 1262, 1119. MS (FAB/NBA): *m*/*z* = 375 [M$^+$] (100%).

***N*1-2-[2-(2-[(3a*R*,8b*S*)-3-((*E*)-1-[(2*S*)-4-Methyl-5-oxo-2,5-dihydro-2-furanyl]oxymethylidene)-2-oxo-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]aminocarbonylaminoethoxy)-ethoxy]ethyl-5-(2-oxoperhydrothieno[3,4-*d*]imidazol-4-yl)-pentanamide (+)-14**

Complete deprotection of (+)-**7** (46 mg, 0.11 mmol) was achieved with 1.5 mL TFA and 10 mL CH_2Cl_2 after 1 h. After evaporation to remove the excess acid, EtOAc was added and pH 8 was adjusted by addition of saturated $NaHCO_3$. The organic layer was then washed with a little cold water and dried ($MgSO_4$). Volatiles were evaporated and the free amine was dissolved in dry CH_2Cl_2 (20 mL). Then 1.05 equiv. of *p*-nitrophenyl chloroformate (23 mg, 0.115 mmol) and 1.05 equiv. of pyridine (10 μL, 0.115 mmol) were added and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with $CHCl_3$ (100 mL), washed with 1% $KHSO_4$ and dried ($MgSO_4$) to obtain (42 mg, 80%) of yellow solid after recrystallization from ethyl acetate–hexane. To a solution of crude carbamate (17 mg, 0.048 mmol) in 2 mL $CHCl_3$, **13** (13 mg, 0.048 mmol) and Et_3N (6.7 μL, 0.048 mmol) were added. TLC control ($CHCl_3$–MeOH 5 : 1) after 15 min showed complete conversion. Solvent was removed *in vacuo*. Purification by column chromatography (SiO_2, $CHCl_3$–MeOH 7 : 1) gave 32 mg of product (+)-**14** as a white solid (64% yield) after precipitation from diethyl ether. TLC: ($CHCl_3$–MeOH 5 : 1, R_f = 0.44). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (m, 6H, biotin-CH_2), 2.03 (s, 3H, CH_3-Dring), 2.06 (t, 2H, J = 7.5 Hz, CH_2CO), 2.70 (d, 1H, J = 12.6 Hz, CHH_{endo}S), 2.84 (dd, 1H, J_1 = 12.6 Hz, J_2 = 4.5 Hz, 1H, CHH_{exo}S), 3.06 (m, 2H, CHS and H4), 3.33 (dd, 1H, J_1 = 16.8 Hz, J_2 = 9.3 Hz, H4), 3.44 (m, 4H, OCH_2), 3.55 (m, 2H, CH_2NH), 3.62 (s, 4H, OCH_2CH_2O), 3.91 (m, 1H, *H*3a), 4.23, 4.46 (m, 2H, CHNH), 5.29 (s, 1H, NH), 5.88 (d, J_1 = 7.8 Hz, 1H, *H*8b), 5.97 (br t, 1H, NH), 6.21 (s, 1H, *H*2′), 6.33, 6.79 (s, 2H, NH), 6.99 (m, 1H, *H*3′), 7.11 (d, 1H, J = 9.0 Hz, *H*5), 7.40 (m, 1H, *H*8), 7.52 (d, 1H, J = 2.4 Hz, *H*6′), 7.60 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz, *H*6), 8.43 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 174.18, 171.8, 170.9, 163.97, 156.25 (5C, *C*O), 151.58 (1C, *C*H6′), 141.1 (1C, *C*H3′), 139.64, 139.28, 135.77, 135.69 (4C, 3Cq_{arom}, Cq D-ring), 125.37, 121.38, 116.16 (3C, *C*H$_{arom}$), 112.96 (1C, *Cq*C-ring), 100.73 (1C, *C*H2′), 86.15 (1C, *C*H8b), 70.71, 70.52, 69.75, 69.68 (4C, *C*H$_2$O), 61.79, 60.13 (2C, *C*HNH), 55.41 (1C, *C*HS), 40.44 (1C, *C*H$_2$S), 39.72 (1C, *C*H3a), 39.41, 39.29 (2C, *C*H$_2$NH), 36.55 (1C, *C*H$_2$), 35.98 (1C, *C*H$_2$CO), 28.05, 27.91, 25.48 (3C, *C*H$_2$), 22.85 (1C, *C*H$_3$ D-ring); Anal. calcd. for $C_{34}H_{43}N_5O_{10}S$: C, 57,21; H, 6,07; N, 9,81 found: C, 60.36; H, 5.73; N, 7.90%.

N1-2-[2-(2-[(2-Oxo-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-7-yl)aminocarbonylamino]ethoxy)ethoxyethyl]-5-(2-oxo-perhydrothieno[3,4-*d*]imidazol-4-yl)pentanamide 17

Compound **17** was prepared in the same way as (+)-**14**, starting from *rac*-**5** (100 mg, 0.34 mmol). Purification by column chromatography (SiO_2, $CHCl_3$–MeOH 7 : 1) gave 23 mg of product **17** as a white solid (89% yield calculated on the carbamate) after precipitation from diethyl ether. TLC: ($CHCl_3$–MeOH 5 : 1, R_f = 0.44). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (m, 6H, biotin-CH_2), 2.21 (t, 2H, J = 7.5 Hz, CH_2CO), 2.37 (ddd, J_1 = 20 Hz, J_2 = 4.9 Hz, J_3 = 2.4 Hz, *H*3), 2.72 (dd, 1H, J_1 = 12.6 Hz, J_2 = 3.2 Hz, CHH_{endo}S), 2.84 (dd, 1H, J_1 = 12.6 Hz, J_2 = 4.5 Hz, 1H, CHH_{exo}S), 2.88 (dd, J_1 = 18 Hz, J_2 = 9.8 Hz, *H*3), 3.09 (m, 2H, CHS and *H*4), 3.21 (dd, 1H, J_1 = 16.4 Hz, J_2 = 8.5 Hz, *H*4), 3.33 (m, 1H, *H*3a), 3.44 (m, 4H, OCH_2), 3.56 (m, 2H, CH_2NH), 3.62 (s, 4H, OCH_2CH_2O), 4.26, 4.48 (m, 2H, CHNH), 5.55, 5.57 (2s, 1H, NH), 5.81 (d, J_1 = 7.5 Hz, 1H, *H*8b), 6.01 (t, J = 4.9 Hz, 1H, NH), 6.59, 6.69 (s, 2H, NH), 6.94 (m, 1H), 7.13 (d, 1H, J = 8.3 Hz, H5), 7.40, 7.42 (2m, 1H, *H*8), 7.60 (dd, 1H, J_1 = 9.3 Hz, J_2 = 1.9 Hz, *H*6), 8.37 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 177.32, 174.18, 163.97, 156.25 (5C, *C*O), 139.63, 139.18, 135.67 (3C, 3Cq_{arom}), 125.54, 121.14, 116.16 (3C, *C*H$_{arom}$), 87.99 (1C, *C*H8b), 70.65, 70.41, 69.75, 69.68 (4C, *C*H$_2$O), 61.75, 60.22 (2C, *C*HNH), 55.49 (1C, *C*HS), 40.45 (1C, *C*H$_2$S), 39.71, 39.36 (2C, *C*H$_2$NH), 37.65 (1C, *C*H3a), 37.33 (1C, *C*H$_2$), 35.91 (1C, *C*H$_2$CO), 35.77 (1C, *C*H$_2$CO), 28.01, 27.90, 25.50 (3C, *C*H$_2$); Anal. calcd. for $C_{28}H_{39}O_7N_5S$–H_2O: C, 55.26; H, 6.80; N, 11.51 found: C, 55.35; H, 7.03; N, 11.17%.

***tert*-Butyl *N*-(2-{[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*SR*) furanyl]oxy}methylidene)-2-oxo-3,3a(*RS*),4,8b(*SR*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]amino}-2-oxoethyl)-carbamate *rac*-18**

Protected Boc-amino-GR24 *rac*-**8** (150 mg, 0.36 mmol) was dissolved in a mixture of trifluoroacetic acid (1 mL) and dichloromethane (10 mL) and stirred at room temperature for 3 h. When TLC analysis indicated complete deprotection, solvents were evaporated *in vacuo*. The residue was suspended in dichloromethane (5 mL) and treated with triethylamine (300 μL). After washing with satd. NaCl (1×) and drying ($MgSO_4$), volatiles were evaporated and the free amine was dissolved in THF (10 mL). Then 1.05 equiv. of 2,3,4,5,6-pentafluorophenyl 2-[(*tert*-butoxycarbonyl)amino]acetate (BocGly-OPfP, 130 mg, 0.38 mmol) and 1.05 equiv. of hydroxybenzotriazole (HOBT, 52 mg, 0.38 mmol) were added and the mixture was stirred at room temperature for 3 h. THF was removed *in vacuo* and the residue was purified over silica gel (ethyl acetate–hexane 3 : 1) to give *rac*-**18** as a white solid (161 mg, 95%) that was recrystallized from dichloromethane–ethyl acetate. Mp: 206–208 °C. ^{1}H NMR (300 MHz,$CDCl_3$): δ 1.47 (s, 9H, 3 × CH_3 tBu), 2.05 (s, 3H, CH_3 D-ring), 3.04 (dd, 1H, $J_{4,\ 3a,\ cis}$ = 2.6 Hz, ^2J = 16.8 Hz, H4), 3.36 (dd, 1H, $J_{4,\ 3a,\ trans}$ = 9.3 Hz, ^2J =16.8 Hz, H4), 3.93 (m, 2H, H3a + CH_2 Gly), 5.29 (br s, 1H, NH), 5.90 (d, 1H, J = 7.9 Hz, H8b), 6.18 (m, 1H, H2′), 6.97 (m, 1H, H3′), 7.17 (d, 1H, J = 8.2 Hz, H5), 7.49 (d, 1H, $J_{3a,6}$ = 2.4 Hz, H6′), 7.52 (dd, 1H, J = 8.3 Hz, $^4J_{6,\ 8}$ = 1.5 Hz, H6), 7.62 (d, 1H, ^4J = 1.5 Hz, H8), 8.43 (br s, 1 H, NH). ^{13}C NMR ($CDCl_3$): δ 11.4 (CH_3 D-ring), 29.0 (CH_3 tBu), 37.6 ($CH_2$4), 39.9 (CH3a), 46.6 (CH_2 Gly), 81.3 (Cq tBu), 86.5 (CH8b), 101.4 (CH2′), 113.9 (Cq C-ring), 118.4, 123.1, 126.3 (3 × CH_{arom}), 136.6, 137.7, 139.2, 140.2 (3 × $Cq_{arom}$and Cq D-ring), 141.7 (CH3′), 151.6 (CH6′), 153.4 (C=O carbamate), 168.6 (C=O gly), 170.9, 171.9 (2 × C=O lactones). IR (KBr): ν/cm^{-1} 3346, 3282 (NH), 1788, 1742, 1721, 1682 (4 × C=O, C=C), 1183 (lactone). MS [FAB *m*/*z*, rel. intensity (%)]: 493 ([M + Na]$^+$, 54.3), 470 ([M]$^+$, 12.4), 371 ([$C_{19}H_{19}O_6N_2$]$^+$, 59.3), 97 ([$C_2H_5O_2$]$^+$, 100). Anal. calcd. for $C_{24}H_{26}O_8N_2$: C, 61.27; H, 5.57; N, 5.95 found: C, 61.06; H, 5.43; N, 5.93%.

***tert*-Butyl *N*-(2-{[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*S*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]amino}-2-oxoethyl)carbamate (−)- 18**

Compound (−)-**18** was prepared in the same way as described for *rac*-**18** starting from carbamate (−)-**8** (200 mg, 0.48 mmol). Yield: 97%. Recrystallization from dichloromethane–ethyl acetate gave a white fluffy solid. Mp: 189–190 °C. $[a]_D^{22}$= −298.9 (c = 0.1, CH_2Cl_2). Anal. calcd. for $C_{24}H_{26}O_8N_2$: C, 61.27; H, 5.57; N, 5.95 found: C, 61.03; H, 5.55; N, 5.92%. All other analytical data were the same as reported for its racemate *rac*-**18**.

4-[({(2-{[3-((*E*)-1-{[4′-Methyl-5′-oxo-2′,5′-dihydro-2′(*S*) furanyl]oxy}methylidene)-2-oxo-3,3a(*R*),4,8b(*S*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]amino}-2-oxoethyl]amino}carbothioyl)amino]-2-[6-(dimethylamino)-3-(1,1-dimethylammonio)-3*H*-9-xanthenyl]benzoate (−)-19

The amine protecting group was removed from carbamate (−)-**18** (16 mg, 0.034 mmol) by stirring for 1 hour at room temperature in a mixture of dichloromethane (2 mL) and

trifluoroacetic acid (0.25 mL). Subsequently the mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane (0.5 mL) and triethylamine (100 mL) was added to liberate the free amine. Tetramethylrhodamine isothiocyanate (15 mg, 0.034 mmol) was dissolved in DMF (2 mL) and added to the solution of deprotected (−)-**18**. The desired thiourea linkage was formed instantly, as indicated by TLC analysis. After stirring for 1 hour at room temperature, solvents were evaporated *in vacuo*, and the residue was purified by flash chromatography over silica gel (10–50% methanol in dichloromethane) yielding a bright pink solid (14 mg, 53%). Mp: >300 °C. $[\alpha]_D^{22} = -270.6$ (c = 0.03, H_2O). ^{1}H NMR (300 MHz, DMSO-d_6): d 1.92 (s, 3H, CH_3 D-ring), 2.88 (m, 1H, H4), 2.94 (s, 12H 4 CH_3TRITC), 3.30 (m, 1H, H4), 3.94 (m, 1H, H3a), 4.30 (br s, 2H, CH_2Gly), 5.97 (d, 1H, J = 7.9 Hz, H8b), 6.49–6.58 (m, 6H, NH thiourea + 4H_{arom}TRITC), 6.69 (m, 1H, H2′), 6.82 (d, 1H, J = 8.3 Hz, H_{aroma}TRITC), 7.14 (d, 1H, J = 8.3 Hz, H_{aroma}TRITC), 7.22 (d, 1H, J = 8.3 Hz, H5), 7.40 (m, 1H, H3′), 7.46 (d, 1H, J = 8.3 Hz, H6), 7.70 (d, 1H, $^4J_{3a,\,6'}$ = 2.4 Hz, H6′), 7.75 (br s, 1H, H8), 7.80 (d, 2H, J = 7.9 Hz, H_{arom}TRITC), 7.89 (d, 1H, J = 8.7 Hz, H_{arom}TRITC), 10.2 (s, 1H, NH_{arom}). ^{13}C NMR (DMSO-d_6): d 10.2 (CH_3D-ring), 36.3 ($CH_2$4), 38.6 (CH3a), 39.8 (CH_3TRITC), 47.4 (CH_2Gly), 85.2 (CH8b), 101.3 (CH_2′), 106.3 (2 × CqTRITC), 111.7 (CqC-ring), 97.9, 108.3, 113.8, 116.2, 121.1, 125.5, 120.6, 121.0, 124.9, 125.5, 127.4, 128.3 (12 × CH_{arom}), 133.7 (CqD-ring), 137.3, 138.0, 139.7 (3 × Cq_{arom},GR24), 143.2 (CH3′), 151.8, 152.1 (6 × CqTRITC), 152.8 (CH6′), 153.8 (Cq–COO^-), 167.9, 168.4, 170, 170.6 (4C, C=O), 180.1 (1C, C=S). IR (KBr): ν/cm^{-1} 3422 (CO*OH*), 3299 (NH), 2989 (CH_{arom}), 1783, 1749, 1680, 1648 (5 × C=O, C=C), 1536 (amide II). MS [FAB *m*/*z*, rel. intensity (%)]: 836 ([M + Na]$^+$, 7.3), 814 ([M + H]$^+$, 13.7), 414 ([C_{20}-$H_{18}O_6N_2S$]$^+$, 100).

9*H*-9-Fluorenylmethyl *N*-[2-{[3-((*E*)-1-{[4′-methyl-5′-oxo-2′,5′-dihydro-2′(*SR*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*RS*),-4,8b(*SR*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]amino}-1-({[(acetylamino)methyl]sulfanyl}methyl)-2-oxoethyl]carbamate] *rac*-20

Racemic, slow moving Boc-amino-GR24 diasteroisomer *rac*-**8** (829 mg, 2.0 mmol) was deprotected by stirring for 1 hour at room temperature in a mixture of dichloromethane (9 mL) and trifluoroacetic acid (1 mL). Then solvents were evaporated under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and triethylamine (1 mL) was added to liberate the free amine. The mixture was washed with brine (1×), dried ($MgSO_4$) and concentrated. After dissolving the residue in THF (10 mL), 1.1 equiv. of Fmoc-and Acm-protected pentafluorophenyl ester of cysteine (FmocCys(Acm)-Opfp, 1.28 g, 2.2 mmol) and 1.1 equiv. of hydroxybenzotriazole (HOBT, 300 mg, 2.2 mmol) dissolved in THF (3 mL) were added. The mixture was stirred at room temperature for 3 hours. THF was removed *in vacuo* and the residue was dissolved in ethyl acetate (20 mL), washed with brine (1×), dried ($MgSO_4$) and concentrated *in vacuo*. Silica gel chromatography (dichloromethane–methanol 15 : 1) gave *rac*-**20** as a white solid (1.39 g, 98%). An analytically pure sample was obtained by recrystallization from ethyl acetate–diisopropyl ether. Mp: 204–206 °C. ^{1}H NMR (300 MHz, DMSO): δ 1.86 (s, 3H, CH_3Acm), 1.92 (s, 3H, CH_3 D-ring), 2.77–2.99 (m, 3H, CH_2 cys + H4), 3.30 (m, 1H, H4), 3.94 (m, 1H, H3a), 4.21–4.40 (m, 8H, CH cys + CH_2cys + CH Fmoc + CH_2Fmoc + CH_2Acm), 5.98 (d, 1H, J = 7.9 Hz, H8b), 6.70 (m, 1H, H2′), 7.24 (d, 1H, J = 8.3 Hz, H5), 7.30–7.90 (m, 10H, NHcys + H3′ + 8H_{arom}Fmoc), 7.70 (d, 1H, $^4J_{3a,\,6'}$ = 2.3 Hz, H6′), 7.74 (d, 1H, J = 8.3 Hz, H6), 7.87 (br s, 1H, H8), 8.58 (t, 1H, J = 6.2 Hz, NHAcm), 10.10 (s, 1H, NHarom). ^{13}C NMR (DMSO): δ 10.2 (CH_3D-ring), 22.6 (CH_3Acm), 32.3 (CH_2cys), 36.3 ($CH_2$4), 38.6 (CH3a), 40.2 (CH_2Acm), 46.6 (CHFmoc), 65.8 (CH_2 Fmoc), 85.2 (CH8b), 101.3 (CH2′), 111.7 (CqC-ring), 116.4, 121.1, 125.5 (3 × CH_{arom}-GR24), 120.1, 125.3, 127.1, 127.6 (8 times; CH_{arom}Fmoc), 133.7 (CqD-ring), 137.4, 138.0, 139.7 (3 × Cq_{arom},GR24), 140.7, 143.8 (4 × CqFmoc), 143.2 (CH3′), 152.8 (CH6′), 156.0 (C=O Fmoc), 169.4, 169.8, 170.7, 170.8 (4 × C=O). IR (KBr): ν/cm^{-1} 3327 (NH), 3066 (CH_{arom}), 2942 (CH_{aliph}), 1795, 1770, 1736, 1709, 1695, 1679 (5 × C=O, C=C), 1535 (amide II). MS [FAB *m*/*z*, rel. intensity (%)]: 732 ([M + Na]$^+$, 28.2), 710 ([M + H]$^+$, 15.3). Anal. calcd. for $C_{38}H_{35}O_9N_3S$: C, 64.30; H, 4.97; N, 5.92; S, 4.52 found: C, 63.83; H, 4.94; N, 5.70; S, 4.26%.

N-1-(2-{[3-((*E*)-1-{[4′-Methyl-5′-oxo-2′,5′-dihydro-2′(*SR*)-furanyl]oxy}methylidene)-2-oxo-3,3a(*RS*),4,8b(*SR*)-tetrahydro-2*H*-indeno[1,2-*b*]furan-7-yl]amino}-2-oxoethyl)-4-azido-2-hydroxybenzamide *rac*-23

Compound *rac*-**18** (39 mg, 0.083 mmol) was dissolved in a mixture of dichloromethane (2 mL) and trifluoroacetic acid (0.25 mL) and stirred at room temperature for 2 hours. When TLC indicated complete removal of the Boc protective group, solvents were evaporated *in vacuo*. The residue was dissolved in THF (2 mL) and triethylamine (100 μL) was added which was immediately followed by the addition of 1 : 1 equiv. of azido salicylic acid succinimidyl ester **22** (25 mg, 0.090 mmol), dissolved in THF (0.5 mL). The reaction takes place instantaneously, as was indicated by TLC analysis, and a white solid appeared in the solution. According to TLC analysis, this solid was excess ester **22**, which was removed by filtration. THF was evaporated *in vacuo*. The residue was dissolved in ethyl acetate, washed with aqueous $NaHCO_3$ (5%) to remove *N*-hydroxysuccinimide, dried ($MgSO_4$) and concentrated. Upon concentration, *rac*-**23** (44 mg, 99%) was obtained as white solid, which was recrystallized from ethyl acetate–hexane. Mp: 200 °C. ^{1}H NMR (300 MHz, DMSO): δ 1.92 (s, 3H, CH_3 D-ring), 2.87 (dd, 1H, $J_{4,3a\ cis}$ = 2.5 Hz, ^2J = 16.7 Hz, H4), 3.30 (m, 2H, H4 + OH), 3.94 (m, 1H, H3a), 4.11 (m, 2H, CH_2 Gly), 5.97 (d, 1H, J = 7.9 Hz, H8b), 6.63 (d, 1H, ^4J = 2.1 Hz, –CN_3CHCOH–), 6.70 (m, 1H, H2′), 6.70 (dd, 1H, J_1 = 8.6 Hz, ^4J = 2.1 Hz, –CN_3CHCH–), 6.97 (m, 1H, H3′), 7.23 (d, 1H, J = 8.3 Hz, H5), 7.49 (d, 1H, J = 8.3 Hz, H6), 7.70 (d, 1H, $^4J_{3a,\,6'}$ = 2.3 Hz, H6′), 7.79 (br s, 1H, H8). 7.95 (d, 1H, J = 8.6 Hz, CN_3CH*CH*–), 9.41 (br s, 1H, NH Gly), 10.18 (S, 1H, NH_{arom}). ^{13}C NMR (DMSO): δ = 10.2 (CH_3 D-ring), 36.4 ($CH_2$4), 38.5 (CH3a), 43.0 (CH_2 Gly), 85.2 (CH8b), 101.3 (CH2′), 111.8 (Cq_{arom} C-ring), 112.7 (Cq_{arom}), 107.0, 110.0, 116.3, 121.0, 125.5, 130.3 (6 × CH_{arom}), 133.7 (CqD-ring), 137.3, 138.1, 139.7 (3 × Cq_{arom},GR24), 143.2 (CH3′), 144.5 (Cq_{arom}-N_3), 152.8 (CH6′), 160.7 (Cq_{arom}-OH), 167.3, 167.9, 170.6, 170.8 (4 × C=O). IR (KBr): ν/cm^{-1} 3575 (OH), 3315 (NH), 1780, 1742, 1728, 1700, 1689 (4 × C=O, C=C). MS [FAB *m*/*z*, rel. intensity (%)]: 554 ([M + Na]$^+$, 24.6), 532 ([M + H]$^+$, 32.3), 97 ([$C_5H_5O_2$]$^+$, 51.1). Anal. calcd. for $C_{26}H_{21}O_8N_5$: C, 58.76; H, 3.98; N, 13.18 found: C, 57.99; H, 3.99; N, 12.94%.

X-Ray crystallography ‡

Crystals of (+)-**8**, suitable for X-ray diffraction studies, were obtained by slow evaporation from 2-methyl-2-butanol (see Table 2).

Bioassays

Plant material. Seeds of *Striga hermonthica* (Del.) Benth. were collected from Sorghum (Sorghum bicolor (L.) Moench) on Gezira Research station, Sudan in 1994. The seeds were stored in glass vials in the dark at room temperature until use in germination tests.

‡ CCDC reference number(s) 197746. See http://www.rsc.org/suppdata/ob/b2/b210678g/ for crystallographic files in .cif or other electronic format.

Table 2 Crystal data and structure refinement for slow moving BocNH-GR24 diastereoisomer (+)-**8**

Empirical formula	$C_{22}H_{23}NO_7$
Formula weight	413.41
Crystal colour	Transparent colourless
Crystal shape	Regular rod
Crystal size	0.42 × 0.12 × 0.11 mm
Temperature	293(2) K
Radiation/wave length	Cu-Kα (graphite monochrom.)/ 1.54184 Å
Crystal system/space group	Orthorhombic/$P2_12_12_1$
Unit cell dimensions	a = 9.3201(4) Å
(25 reflections 22.177 < θ < 45.115)	b = 12.5782(4) Å
	c = 17.9679(4) Å
Volume	2106.38(11) Å^3
Z/calculated density absorption coefficient diffractometer/scan	4/1.304 mg m^{-3}/ 0.815 mm^{-1} Enraf-Nonius CAD4/θ–2θ
F(000)	872
θ range for data collection	4.29 to 69.94
Index range	$0 \le h \le 11$, $0 \le k \le 15$, $0 \le k \le 21$
Reflections collected/unique	2282/2282
Reflections observed	1967 ([$I_0 > 2\sigma(I_0)$])
Absorption correction	Semi-empirical from Ψ-scans
Range of relat. transm. factors	1.055 and 0.960
Refinement method	Full-matrix least squares on F^2
Computing	SHELXL-97[31]
Data/restraints/parameters	2282/0/364
Goodness-of-fit on F^2	1.048
SHELXL-97 weight parameters	0.077600 and 0.140500
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0398, wR_2 = 0.1093
Final R indices (all data)	R_1 = 0.0485, wR_2 = 0.1169
Extinction coefficient	0.0023(4)
Largest diff. peak and hole	0.192 and −0.178 e Å^3

Preparation of test solutions. A compound to be tested was weighed out very accurately to the amount of 1.0 mg, dissolved in 5 mL acetone p.a. and diluted with demineralized water to 50 mL. These stock solutions of approximately 10^{-4} mol L^{-1} (the exact concentration depending on the molecular mass of the compound used) were further diluted with demineralized water to obtain test solutions with concentrations ranging from 2×10^{-5} and 2×10^{-7} mol L^{-1}. All solutions were prepared just before use.

Bioassays. All bioassays were performed at the Department of Organic Chemistry University of Nijmegen The Netherlands in 1999 and 2001. For surface sterilization all seeds were exposed for 5 minutes to 50% (v/v) aqueous solutions of commercial bleach (2% hypochlorite). Subsequently, the seeds were thoroughly rinsed with demineralized water and air-dried. For conditioning the seeds were spread on glass fiber filter paper disks (8 mm diameter, approximately 60–100 seeds per disk) in Petri dishes, wetted with demineralized water and stored in the dark at 30 °C. Thereafter the conditioning water was removed and conditioned seeds were placed in new Petri dishes and exposed to test solution. After incubation for 24 hours in the dark at the indicated temperatures the percentages of germination seeds were determined under a microscope. Seeds were considered to be germinated if the radicle protruded through the seed coat. In each test series an aqueous solution of 0.1% acetone was included as a negative control. For full details of the bioassays, see Mangnus *et al.*[36]

References

1 (*a*) *Parasitic Weeds in Agriculture.*, ed. L. J. Musselman, Vol. I, Striga; CRC Press: Boca Raton, FL, USA, 1987; (*b*) D. M. Joel and V. H. Portnoy, *Ann. Bot. (London)*, 1998, **81**, 779.
2 C. E. Cook, L. P Whichard, B. Turner, M. E. Wall and G. H. Egley, *Science*, 1966, **154**, 1189.
3 C. Hauck, S. Muller and H. Schildknecht, *J. Plant Physiol.*, 1992, **139**, 474.
4 T. Yokota, H. Sakai, K. Okuno, K. Yoneyama and Y. Takeuchi, *Phytochemistry*, 1998, **49**, 1967.
5 S. Muller, C. Hauck and H. Schildknecht, *J. Plant Growth Regul.*, 1992, **11**, 77.
6 C. Hauck and H. Schildknecht, *J. Plant Physiol.*, 1990, **136**, 126–128.
7 C. Bergmann, K. Wegmann, K. Frischmuth, E. Samson, A. Kranz, D. Weigelt, P. Koll and P. Welzel, *J. Plant Physiol.*, 1993, **142**, 338.
8 Y. Sugimoto, S. C. M. Wigchert, J. W. J. F. Thuring and B. Zwanenburg, *J. Org. Chem.*, 1998, **63**, 1259.
9 (*a*) A. Reizelman, M. Scheren, G. H. L. Nefkens and B. Zwanenburg, *Synthesis*, 2000, 1944; (*b*) A. Reizelman and B. Zwanenburg, *Synthesis*, 2000, 1952.
10 (*a*) E. Magnus and B. Zwanenburg, *J. Agric. Food Chem.*, 1992, **40**, 69; (*b*) J. W. J. F. Thuring, G. H. L. Nefkens, R. Schaafstra and B. Zwanenburg, *Tetrahedron*, 1995, **51**, 5047.
11 J. W. J. F. Thuring, G. H. L. Nefkens and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 2278.
12 G. H. L. Nefkens, J. W. J. F. Thuring, M. F. M. Beenakkers and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 2273.
13 S. C. M. Wigchert and B. Zwanenburg, *J. Agric. Food Chem.*, 1999, **47**, 1320.
14 E. M. Magnus, L. A. van Vliet, D. A. L. Vandenput and B. Zwanenburg, *J., Agric. Food Chem.*, 1992, **40**, 1066.
15 J. W. J. F. Thuring, N. W. J. T. Heinsman, R. W. A. W. M. Jacobs, G. H. L. Nefkens and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 507.
16 A. W. Johnson, G. Roseberry and C. Parker, *Weed Res.*, 1976, **16**, 223.
17 S. C. M. Wigchert, E. Kuiper, G. J. Boelhouwer, G. H. L. Nefkens, J. A. C. Verkleij and B. Zwanenburg, *J. Agric. Food Chem.*, 1999, **47**, 1705.
18 N. M. Green, L. Konieczny, E. J. Toms and R. C. Valentine, *Biochem. J.*, 1971, **125**, 781.
19 (*a*) A. W. Johnson, G. Gowda, A. Hassanali, J. Knox, S. Monaco, Z. Razawi and G. Roseberry, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1734; (*b*) J. W. J. F. Thuring, G. H. L. Nefkens and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 2278; (*c*) E. M. Magnus, F. J. Dommerholt, R. L. P. de Jong and B. Zwanenburg, *J., Agric. Food Chem.*, 1992, **40**, 1230.
20 J. W. J. F. Thuring, R. Keltjens, G. H. L. Nefkens and B. Zwanenburg, *J. Chem. Soc., Perkin. Trans. 1*, 1997, 759.
21 A. J. W. G. Visser and M. Hink, *J. Fluoresc.*, 1999, **9**, 81.
22 S. Sterrer and K. Henco, *J., Recept. Transduction Res.*, 1997, **17**, 511.
23 E. W. Van der Vegte and G. Hadziioannou, *Langmuir*, 1997, **12**, 4357.
24 H. Bayley, *Laboratory techniques in biochemistry and molecular biology*, eds. T. S. Work and R. H. Burdon, Elsevier, Amsterdam, The Netherlands, 1983, pp. 1–187.
25 F. Kotzyba-Hilbert, I. Kapfer and M. Goeldner, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1296.
26 G. B. Schuster and M. S. Platz, *Advances in Photochemistry*, Vol 17. eds. D. Volman, G. Hammond and D. Neckers, Wiley & Sons, New York, USA 1992, p. 69.
27 J. W. J. F. Thuring, H. H. Bitter, M. M. de Kok, G. H. L. Nefkens, A. M. D. A. van Riel and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 2284.
28 K. Frischmuth, U. Wagner, E. Samson, D. Weigelt, P. Koll, H. Meuer, W. S. Sheldrick and P. Welzel, *Tetrahedron: Asymmetry*, 1993, **4**, 351.
29 P. Welzel, S. Röhring and Z. Milkova, *J. Chem. Soc., Chem. Commun.*, 1999, 2017.
30 S. Röhring, L. Henning, M. Findeisen, P. Welzel, K. Frischmuth, A. Marx, T. Petrowitsch, P. Koll, D. Müller, H. Mayer-Figge and W. S. Sheldrick, *Tetrahedron*, 1998, **54**, 3413.
31 V. A. Korshum, N. B. Pestov, E. V. Nozhevnikova, I. A. Prokhorenko, S. V. Gontarev and Y. A. Berlin, *Synth. Commun.*, 1996, **13**, 2531.
32 S. D. Wilbur, D. K. Hamlin, P. M. Pathare and S. A. Weerawarna, *Bioconjugate Chem.*, 1997, **8**, 572.
33 R. P. Haughland, *Handbook of Fluorescent probes and research chemicals.*, Sixth Edition, Molecular Probes, Eugene OR, USA, 1996.
34 *The peptide, analysis, Synthesis & biology* eds. E. Gross and J. Meierhofer, Academic Press, London, UK, 1981, **3**, 137.
35 J. Beekwilder, A. Reizelman, P. Bakker, H. Bouwmeester and B. Zwanenburg, submitted for publication.
36 E. M. Mangnus, P. L. A. Stommen and B. Zwanenburg, *J. Plant Growth Regul.*, 1992b, **11**, 91.