Estrano[17,16-*e*]pyrimidine-amino acid and estrano[17,16-*e*]pyrimidine-peptide conjugates

Tomohiro Matsumoto[a], Kodai Shiine[a], Shuntaro Mataka[b] and Thies Thiemann[a,c,d]

[a]*Interdisciplinary Graduate School of Engineering Sciences and* [b]*Institute of Materials Chemistry and Engineering, Kyushu University, 6-1, Kasuga-koh-en, Kasuga-shi, Fukuoka 816-8580, Japan*

[c]*CIMAGO, University of Coimbra, P-3030 Coimbra, Portugal*

[d]*Present address: College of Science, United Arab Emirates University, Al Ain, United Arab Emirates*

Estrone has been converted to an aminopyrimidino [17,16-e]-annelated estrane derivative. A number of amino acids, dipeptides and tripeptides have been linked to the aminopyrimidino unit of the annelated steroid. The tripeptide motifs may be used for the complexation of metals, such as of technetium as a radiolabel for the potential detection of estrogen positive breast cancer cells.

Keywords: steroids, estranes, breast cancer, diagnostic agents

Recently, a number of estradiol based steroidal metal complexes[1-3] have been proposed as potential radiodiagnostic and therapeutic agents for estrogen receptor positive breast cancer. In these cases, the estradiol derivative interacts with the estrogen receptor, which is found in relatively high concentrations in the cell nuclei of ER-positive breast cancer cells. The use of technetium [^{99m}Tc] as the metal component has been emphasised by a number of research groups.[4,5] Inactive rhenium steroid composites[6,7] have been studied as model compounds for technetium analogues as all isotopes of technetium itself are radioactive. In our own search for suitable radioligands for the estrogen receptor,[8-11] we have recently examined one estrano[17,16-*e*]pyrimidine-tripeptide, Cys–Gly–Cys–aminopyrimidinoestrane **1** (Fig. 1),[12] where the tripeptide was to be utilised as a ligand to bind rhenium or technetium.

The synthesis of three further estranopyrimidine-tripeptides is reported in this paper.

During the last decade, the binding of technetium, especially of the $[TcO]^{3+}$ core, to small peptides has been studied extensively. For this reason, a number of tripeptide motifs are known to function as very suitable ligands for $[TcO]^{3+}$.[13-16] These include Cys–Gly–Cys (**2**), and Me_2Gly–Ser–Cys (**3**) (Fig. 1). The strategy followed here is to link a tripeptide motif to an estradiol based steroid via an aminopyrimidine unit annelated to the estrane skeleton. We have found previously that D-areno annelated estranes show a good biodistribution in estrogen receptor-rich tissues.[10] While this in itself is no indication that aminopyrimidinoestranes will exhibit an equally favourable behaviour *in vivo*, the following constitutes an effort to provide a series of estrane-tripeptide derivatives to be radiolabelled and tested *in vivo* at a later stage. The work is a continuation of the preparation of the aminopyrimidinoestrane–Cys–Gly–Cys motif, reported previously.[12] In the following, the preparations of an aminopyrimidinoestrane linked to the Ser–Gly–Cys–, Ser–Gly–Ser, Gly–Ala–Ser, Ser–Ala–Ser motifs are described.

Suitably protected estrones **6** were converted to 16-hydroxymethylidene-estrones **7** according to a procedure by Tapolcsanyi *et al.*[17,12] As the reactivity of the 17-keto group in

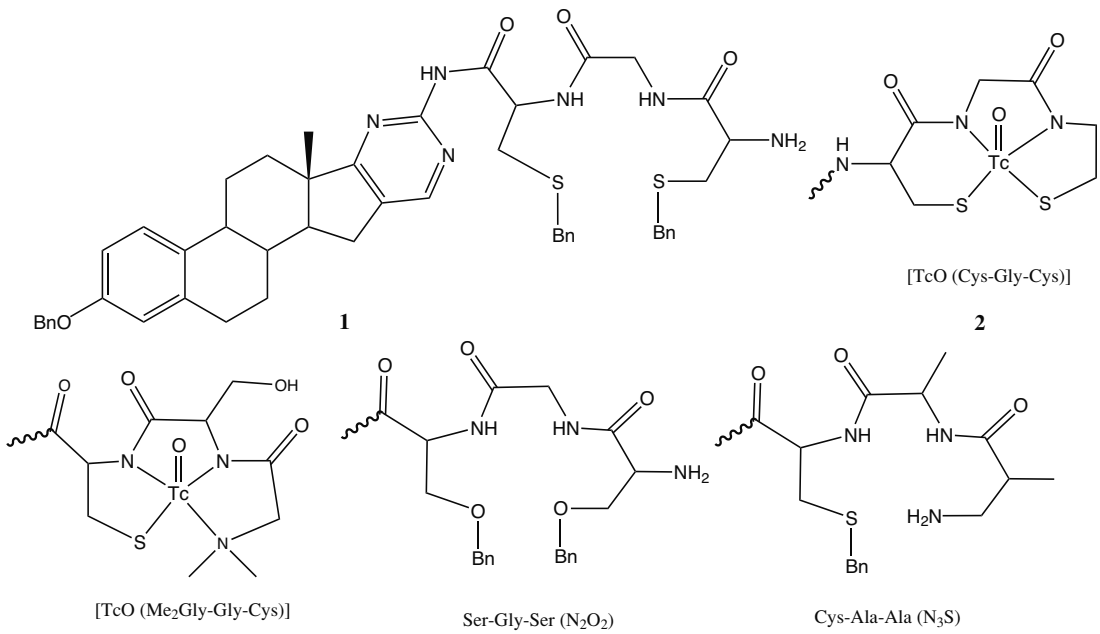
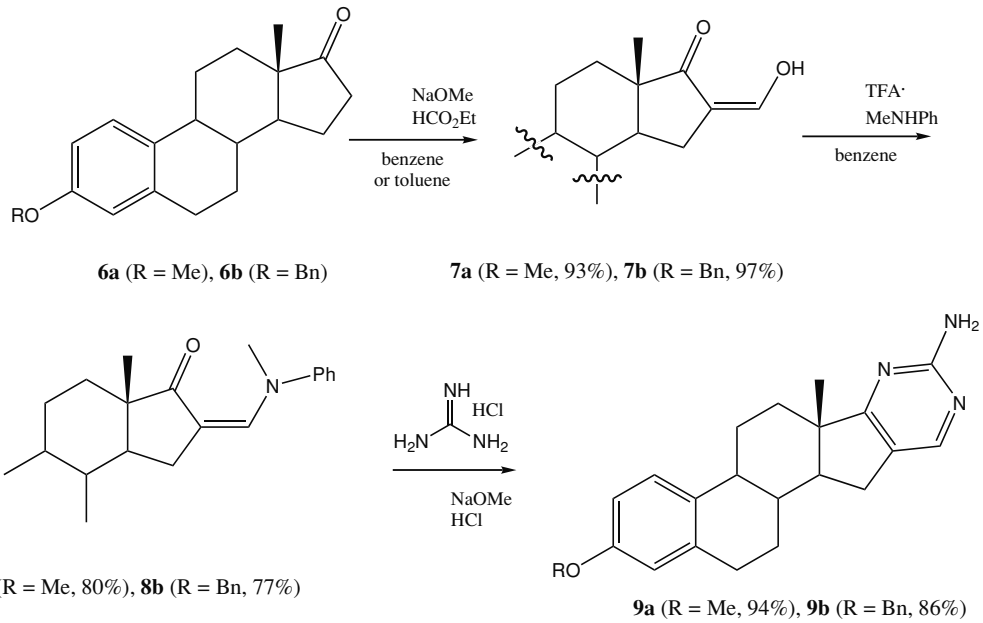


Figure 1 Protected estratetraeno[17,16-*e*]-2'-(Cys–Gly–Cys–amino)pyrimidine (**1**)[12] known tripeptide motifs for $[TcO]^{3+}$, **2** and **3**, and envisaged protected ligands **4** and **5**.

* Correspondent. E-mail: thies@cm.kyushu-u.ac.jp

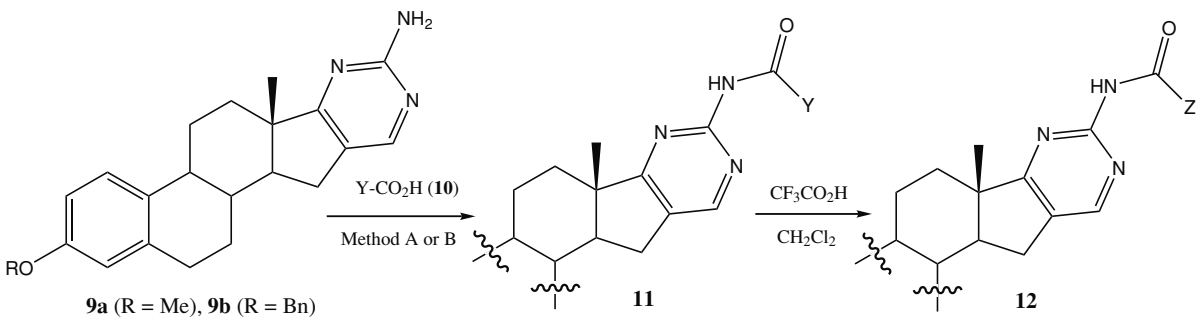


Scheme 1 Preparation of 2'aminopyrimidinoestrances by the general method of Brederick.[18]

7 is low due to the hydrogen bonding with the enol moiety at C-16, compounds **7** were transformed into enamines **8** using *N*-methylphenylammonium trifluoroacetate (Scheme 1).[12] The enamines were reacted with guandiunium hydrochloride (3 equiv.) in the presence of a base according to a general procedure by Bredereck[18] to give the corresponding 2'-aminopyrimidinoestranes **9** in good yield (Scheme 1).[12] Previously, we have carried out an X-ray crystal structural analysis of **9a**[19] that showed that in the crystal the amino-function at C-21 forms a hydrogen bond with N-2. Also, AM1 calculations showed that the electron density at N-2 is higher than at N-1 or at the amino nitrogen. The result indicates that protic interaction will take place predominately at N-2. The combination of N-1, N-2 and the amino function at C-21 gives a high electron density in the vicinity of the amino function of the aminopyrimidinyl moiety. This is important for evaluating the outcome of the coupling reaction with the first amino acid.

Initially, a number of methods were screened for coupling of *N-tert*-butoxycarbonyl protected amino acids **10** to **9** (Scheme 2). These included (a) the traditional DCC method,[20] (b) the use of isobutyl chloroformate (IBCF) and *N*-methylmorpholine (NMM),[21] and (c) the use of 2,2'-dipyridyl disulfide and triphenylphosphine.[22] The introduction of *N-tert*-butoxycarbonylglycine (**10a**) proved to be especially troublesome, giving the desired peptidic coupling product, when using IBCF and NMM, but in poor yield.[12] Nevertheless, for the construction of the peptidoaminopyrimidinoestranes described in this communication, the authors reverted to using the DCC method. Thus, initial experiments coupling of **9b** with *N*-(*tert*-butoxycarbonyl)-(L)-α-aminobutyric acid led to the desired product **11b** in 53% yield. Also, the reaction of **9b** with *N*-(*tert*-butoxycarbonyl)-(*L*)-α-alanine gave the coupling product **11c** in fair yield. Both coupling products were subsequently deprotected with trifluoroacetic acid in dry CH_2Cl_2 at room temperature. Finally, reaction of *N-tert*-butoxycarbonylglycine using the DCC method also gave the coupling product **11a**. However the yield of 25% could not be improved (Scheme 2).

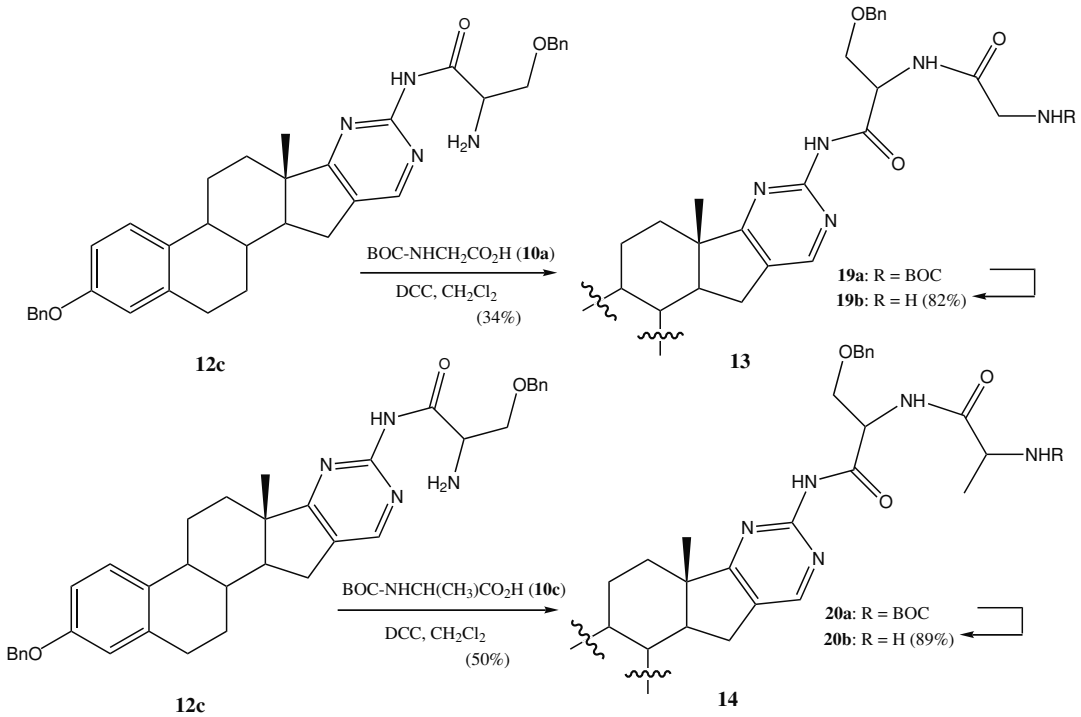
Using the DCC method, serinyl-aminopyrimidinoestrane **12c** was reacted further with BOC-glycine and BOC-(*L*)-alanine to provide the dipeptidylsteroids **13** and **14** (Scheme 3). The



9a (R = Me) Method B **11a**: Y = BOC-NH-CH_2 (25%)
9a (R = Me) Method A **11a**: Y = BOC-NH-CH_2 (25%)
9b (R = Bn) Method A **11b**: Y = BOC-NH(CH)CH_2CH_3 (53%) **12a**: Z = $H_2N(CH)CH_2CH_3$ (77%)
9b (R = Bn) Method A **11c**: Y = BOC-NH(CH)CH_3 (42%) **12b**: Z = $H_2N(CH)CH_3$ (77%)
9b (R = Bn) Method A **11d**: Y = BOC-NH(CH)CH_2OBn (40%) **12c**: Z = $H_2N(CH)CH_2$OBn (96%)

Method A: DCC, CH_2Cl_2; Method B: IBCF, NMM

Scheme 2 Coupling of the first amino acid to the aminopyrimidinoestrane **9**.



Scheme 3 Preparation of aminopyrimidinoestrane-dipeptides **13** and **14**.

previously synthesised[12] cysteinyl-aminopyrimidinoestrane **12e** was also converted to dipeptide **15** (Scheme 4). For the synthesis of the desired steroidal tripeptide conjugates, **13b**, **14b**, and the previously synthesised[12] **16** were reacted further. While for the transformation of **14b** the DCC method was used, **13b** and **16** were reacted with the *N*-hydroxysuccinimide ester of the corresponding amino acid (Scheme 5). Along with the 4-nitrophenyl-, the pentafluorophenyl-, and the *tert*-butyl esters, *N*-hydroxysuccinimides belong to the active ester reagents that circumvent the formation of urea as a by-product. Also, **15b** was reacted with BOC-alanine to tripeptide **21**, using the DCC method (Scheme 6).

In conclusion, a number of aminopyrimidinoestrane-tripeptide conjugates have been prepared successfully. Investigations concerning their metal complexing behaviour are currently underway.

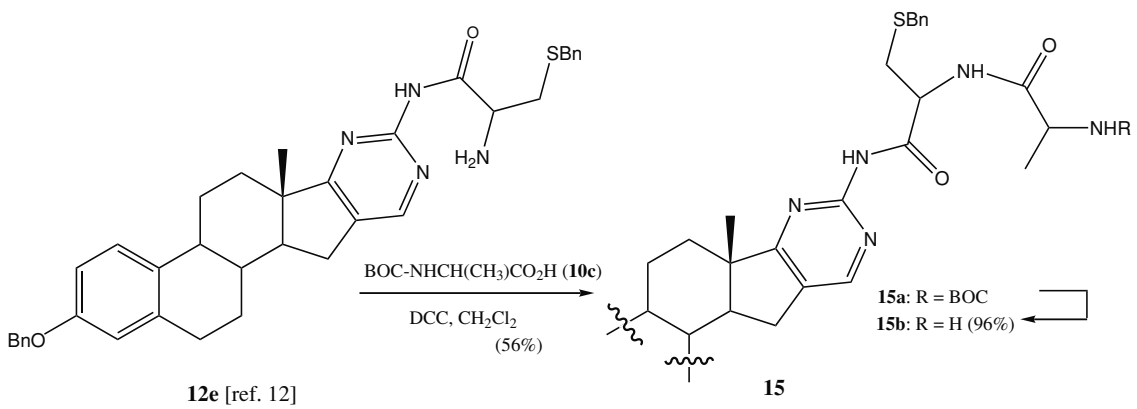
Experimental

General

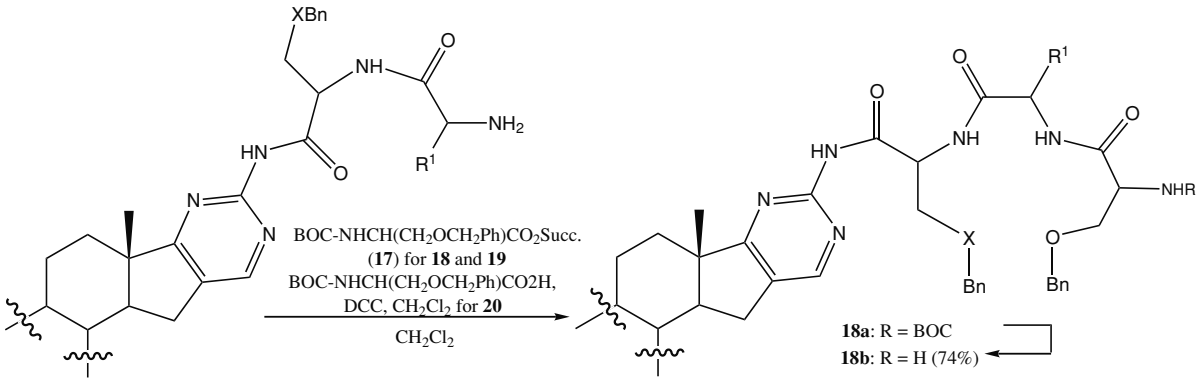
IR spectra were measured with JASCO IR-700 and Nippon Denshi JIR-AQ2OM machines. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL EX-270 spectrometer (^{1}H at 270 MHz and ^{13}C at 67.8 MHz). The chemical shifts are relative to TMS (solvent $CDCl_3$, unless otherwise noted). Mass spectra were measured with a JMS-01-SG-2 spectrometer [electron impact mode (EI), 70 eV or fast atom bombardment (FAB)]. Melting points were measured on a Yanaco microscopic hotstage and are uncorrected. Column chromatography was carried out on Wakogel C-300. All coupling experiments were purged with argon at the start and were carried out under an argon atmosphere.

Starting materials estrone (Wako Pure Chemicals Industries, Ltd.), *N*-(butoxycarbonyl)-*S*-benzyl-*L*-cysteine (Tokyo Kasei Kogyo Co., Ltd), *N*-(butoxycarbonyl)glycine (Wako), *N*-(butoxycarbonyl)-L-alanine (TCI), *N*-(butoxycarbonyl)-*L*-α-aminobutyric acid (Sigma-Aldrich), and *N*-(butoxycarbonyl)-*O*-benzyl-*L*-serine (TCI) were used as purchased. 3-*O*-Methylestrone (KOH, MeI, DMSO)[23] and 3-*O*-benzylestrone (BnBr, NaH, DMF)[24] were synthesised according to known procedures. 3-Methoxy- and 3-benzyloxyestra-1,3,5(10), 16-tetraeno[17,16-e]-2'-aminopyrimidines **9** were synthesised according to a previously published procedure.[12] *N*,*N*-Dimethylformamide and dichloromethane were dried over CaH_2 and toluene was dried over sodium ketyl. Ethyl formate was distilled over phosphorus pentoxide.

3-Methoxyestra-1,3,5(10),16-tetraeno[16,17-e]-2'-(N-tert-butoxy-carbonyl-L-glycylamino)pyrimidine (**11a**); *general method B*: N-Methyl morpholine (NMM) (0.13 mL, 1.19 mmol) and subsequently isobutyl chloroformate (IBCF, 0.08 mL, 0.60 mmol) were added to a solution of **9a** (200 mg, 0.60 mmol) and N-(tert-



Scheme 4 Preparation of aminopyrimidinoestrane-dipeptides **15**.



16: X = S, R^1 = H [ref. 12]
13b: X = O, R^1 = H
14b: X = O, R^1 = CH_3

18: X = S, R^1 = H (56%)
19: X = O, R^1 = R = H (43% over 2 steps, incl. TFA, CH_2Cl_2)
20: X = O, R^1 = CH_3, R = H (33% over 2 steps, incl. TFA)

Scheme 5 Preparation of aminopyrimidinoestrane-tripeptides **18–20**.

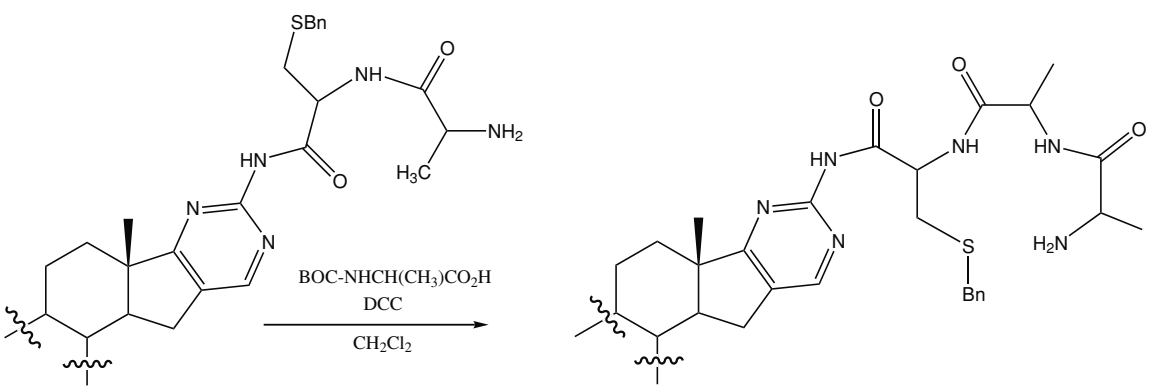
butoxycarbonyl)-L-glycine (**10a**, 157 mg, 0.89 mmol) in dry THF (10 mL). Both additions were carried out at 0 °C, and the resulting mixture was stirred at 0 °C for 1.5 h. CH_2Cl_2 (40 mL) was added to the solution, which was then washed with water (20 mL) and aq. $NaHCO_3$ (30 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/EtOAc 15:1) to give **11a** (74 mg, 25%) as a colourless solid; m.p. 160–161 °C. (Found: MH^+, 493.2814. $C_{28}H_{37}O_4N_4$ requires MH^+, 493.2815). δ_H 1.00 (3H, s, CH_3), 1.48 (9H, s, Bu^t), 3.79 (3H, s, OCH_3), 4.51 (2H, brs), 6.66 (1H, d, ^4J = 2.7 Hz), 6.72 (1H, dd, ^3J = 7.9 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 7.9 Hz), 8.27 (1H, brs, NH), 8.34 (1H, s); δ_C 17.2, 25.0, 26.1, 27.5, 28.4, 29.6, 32.7, 34.0, 37.6, 44.3, 46.5, 55.0, 55.2, 111.6, 114.0, 126.2, 128.8, 132.1, 137.6, 152.6, 155.6, 157.7, 183.6; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 493 (1.2) [MH^+], 492 (2.0) [M^+].

3-Benzyloxyestra-1,3,5(10),16-tetraeno[17,16-e]-2′-(N-tert-butoxycarbonyl-L-α-aminobutyrylamino)pyrimidine (**11b**); *general method A*: *N,N*-Dicyclohexylcarbodiimide (DCC; 350 mg, 1.69 mmol) was added to a solution of 3-benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-*e*]-2'-aminopyrimidine (**9b**, 300 mg, 0.73 mmol) and *N*-(*tert*-butoxycarbonyl)-(L)-α-aminobutyric acid (360 mg, 1.77 mmol) in dry CH_2Cl_2 (15 mL) and the resultant mixture was stirred for 15 h at 40 °C. The precipitated urea was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (EtOAc/Hexane 2:1-EtOAc) to give **11b** (230 mg, 53%) as a colourless solid, m.p. 185–187 °C. (Found: MH^+, 597.3437. $C_{36}H_{45}O_4N_4$ requires MH^+, 597.3441). δ_H 1.00 (3H, s, CH_3), 1.46 (9H, s, Bu^t), 5.04 (2H, s, OCH_2Ph), 6.76 (1H, d, ^4J = 2.4 Hz), 6.81 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (5H, m, Ph), 8.40 (1H, s); δ_C 10.0, 17.2, 24.9, 25.5, 25.9, 26.0, 27.4, 28.3, 29.5, 27.4, 29.5, 31.6, 32.7, 33.8, 37.5, 44.3, 46.5, 54.9, 69.9, 112.4, 114.9, 126.2, 127.4, 127.9, 128.6, 132.3, 137.2, 137.7, 152.7, 155.6, 156.8, 183.5; MS (FAB, 3-nitrobenzylalcohol) *m/z* (%) 597 (0.99) [MH^+] 596 (0.36) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2′-(N-tert-butoxycarbonyl-L-α-alaninylamino)pyrimidine (**11c**): **9b** (300 mg, 0.73 mmol), *N*-(tert-butoxycarbonyl)-(L)-α-alanine (310 mg, 1.63 mmol) and DCC (320 mg, 1.55 mmol) in dry CH_2Cl_2 (15 mL) were reacted (20 h, 40 °C) and subjected to work-up according to method A. Column chromatography on silica gel (EtOAc/hexane 3 : 1-EtOAc) yielded **11c** (179 mg, 42%) as a colourless solid; m.p. 124–125 °C. (Found: MH^+, 583.3284. $C_{35}H_{43}O_4N_4$ requires MH^+, 583.3285). δ_H 1.00 (3H, s, CH_3), 1.46 (9H, s, Bu^t), 3.49 (1H, brs, NH), 5.05 (2H, s, OCH_2Ph), 6.76 (1H, d, ^4J = 2.4 Hz), 6.81 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (5H, m, Ph), 8.39 (1H, s); δ_C 17.1, 18.5, 24.9, 25.6, 26.0, 27.4, 27.5, 28.3, 29.6, 32.7, 33.9, 37.5, 44.3, 46.4, 49.2, 54.9, 69.9, 112.4, 114.9, 126.2, 127.4, 127.9, 128.6, 129.1, 132.4, 137.2, 137.7, 152.7, 155.6, 156.9, 183.5; MS (FAB, 3-nitrobenzylalcohol) *m/z* (%) 583 (10) [MH^+], 582 (2.1) [M^+].

3-Benzyloxyestra-1,3,5(10),16-tetraeno[17,16-e]-2′-(O-benzyl-N-tert-butoxycarbonyl-L-serinylamino)pyrimidine (**11d**): **9b** (1.00 g, 2.43 mmol), (*N-tert*-butoxycarbonyl)-*O*-benzyl-L-serine (1.44 g, 4.86 mmol) and DCC (1.00 g, 4.86 mmol) in dry CH_2Cl_2 (20 mL) were reacted (5 h, r.t.) according to method A. Column chromatography on silica gel (CH_2Cl_2/EtOAc 9:1-2:1-1:1) gave **11d** (665 mg, 40%) as a colourless solid; m.p. 74–76 °C. KBr/cm^{-1} ν_{max} 3734, 3329, 2929, 2852, 2360, 2337, 1701, 1626, 1574, 1506, 1369, 1244, 1165, 669, 420; δ_H (270 MHz) 1.00 (3H, s, CH_3), 1.47 (9H, s, Bu^t), 5.04 (2H, s, OCH_2Ph), 5.05 (2H, s, OCH_2Ph), 6.76 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (10H, m, 2 Ph), 8.41 (1H, s); δ_C 13.7, 16.6, 24.4, 25.1, 25.6, 26.9, 27.1, 27.8, 29.1, 32.3, 33.4, 37.1, 43.8, 45.9, 48.8, 54.4, 58.4, 59.9, 62.1, 69.2, 69.5, 73.1, 111.9, 114.4, 125.7, 127.0, 127.1, 127.4, 127.8, 127.9, 128.0, 128.1, 128.8, 131.9, 136.7, 137.2, 152.1, 155.1, 156.4, 182.9; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 691 (11.5) [MH^+], 690 (24.5) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2′-(L-α-aminobutyrylamino)pyrimidine (**12a**); *general Method C*: **11b** (67.9 mg, 0.114 mmol) was dissolved in dry CH_2Cl_2 (10 mL). Trifluoroacetic acid (0.4 mL, 5.18 mmol) was added to the solution, and the reaction



15b

21: (40% over 2 steps, incl. TFA, CH_2Cl_2)

Scheme 6 Preparation of aminopyrimidinoestrane-tripeptides **21**.

mixture was stirred for 17 h at r.t. CH_2Cl_2 (10 mL) was added to the solution, and thereafter the mixture was washed with aq. Na_2CO_3 (2 × 20 mL) and water (2 × 20 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **12a** (43.7 mg, 77%) as a slowly crystallising, colourless solid. (Found: MH^+, 497.2917. $C_{31}H_{37}O_2N_4$ requires MH^+, 497.2917). δ_H 1.00 (3H, s, CH_3), 1.46 (9H, s, Bu^t), 5.04 (2H, s, OCH_2Ph), 6.76 (1H, d, ^4J = 2.4 Hz), 6.81 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (5H, m, Ph), 8.46 (1H, s); δ_C 10.3, 17.1, 24.9, 24.9, 25.6, 26.0, 27.4, 27.5, 29.6, 32.7, 33.9, 37.5, 44.3, 46.4, 54.8, 69.9, 112.3, 114.8, 126.1, 127.4, 127.9, 128.5, 132.4, 137.2, 137.7, 152.7, 155.9, 156.8, 183.3; MS (FAB, 3-nitrobenzylalcohol) *m/z* (%) 497 (1.3) [MH^+], 496 (0.41) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(L-α-alaninylamino)pyrimidine (**12b**): **11c** (60 mg, 0.124 mmol) and trifluoroacetic acid (0.6 mL, 7.8 mmol) in dry CH_2Cl_2 (7 mL) were reacted (13 h, r.t.) and subjected to work-up according to method C to give **12b** (21 mg, 77%) as a slowly crystallising, colourless solid. (Found: MH^+, 483.2758. $C_{30}H_{35}O_2N_4$ requires MH^+, 483.2760). δ_H 1.00 (3H, s, CH_3), 1.25–2.03 (m, 7H) 1.47 (3H, d, CH_3(ala), ^3J = 6.8 Hz), 2.29–2.58 (4H, m), 2.81–2.97 (3H, m), 3.81 (1H, brs, NH), 5.05 (2H, s, OCH_2Ph), 6.76 (1H, d, ^4J = 2.4 Hz), 6.82 (1H, dd, ^3J = 8.6 Hz ^4J = 2.6 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (5H, m, Ph), 8.45 (1H, s), 10.0 (2H, brs, NH_2); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ_C 17.1, 21.4, 26.0, 27.4, 27.5, 29.5, 32.7, 37.5, 44.3, 46.4, 51.5, 54.8, 69.9, 112.3, 114.8, 126.1, 127.4, 127.9, 128.5, 129.0, 132.4, 137.2, 137.7, 152.7, 155.8, 156.8, 183.3; MS (FAB, 3-nitrobenzylalcohol): *m/z* (%) 483 (MH^+, 12.69), 482 (M^+, 1.45).

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(O-benzyl-L-serinylamino)pyrimidine (**12c**): **11d** (174 mg, 0.25 mmol) and trifluoroacetic acid (2 × [0.24 mL, 3.12 mmol]) in dry CH_2Cl_2 (10 mL) were reacted (1 h, r.t.; then 3 h, r.t.) and subjected to work-up according to method C to give **12c** (143 mg, 96%) as a colourless solid; m.p. 84–86 °C (dec.). (Found: MH^+, 589.3175. $C_{37}H_{41}O_3N_4$ requires MH^+, 589.3179). KBr/cm^{-1} ν_{max} 3327, 2927, 2852, 2360, 1711, 1626, 1576, 1497, 1419, 1311, 1244, 1090, 808, 735, 696, 644; δ_H 1.00 (3H, s, CH_3), 4.57 (2H, s, OCH_2Ph), 5.05 (2H, s, OCH_2Ph), 6.74 (1H, d, ^4J = 2.6 Hz), 6.78 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.25–7.46 (10H, m), 8.45 (1H, s); δ_C 17.1, 24.9, 25.6, 26.1, 27.4, 27.6, 29.6, 32.8, 34.0, 37.5, 44.3, 46.4, 49.1, 54.9, 70.0, 71.9, 73.4, 112.4, 114.9, 126.1, 127.5, 127.7, 127.9, 128.5, 128.6, 129.2, 132.5, 137.2, 137.7, 152.7, 156.7, 156.9, 183.4; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 590 (12.6) [MH^+], 589 (29.9) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(N-tert-butoxycarbonyl-L-glycyl-O-benzyl-L-serinylamino)pyrimidine (**13a**): **12c** (380 mg, 0.65 mmol), N-(*tert*-butoxycarbonyl)-L-glycine (**10a**, 226 mg, 1.29 mmol) and DCC (267 mg, 1.29 mmol) in dry CH_2Cl_2 (25 mL) were reacted (5 h, r.t.) according to method B. Column chromatography on silica gel (CH_2Cl_2/EtOAc 3:1–1:1) gave **13a** (165 mg, 34%) as a slowly crystallising colourless oil. (Found: MH^+, 746.3919. $C_{44}H_{52}O_6N_5$ requires MH^+, 746.3918). KBr/cm^{-1} ν_{max} 3427, 2924, 2856, 2360, 1664, 1500, 1458, 1375, 1248, 1167, 735, 696; δ_H 1.00 (3H, s, CH_3), 1.45 (9H, s, Bu^t), 5.05 (2H, s, OCH_2), 6.76 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (10H, m), 8.40 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 746 (27.5) [MH^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(L-glycyl-O-benzyl-L-serinylamino)pyrimidine (**13b**): **13a** (157 mg, 0.21 mmol) and trifluoroacetic acid [2 × (0.21 mL, 2.73 mmol)] in dry CH_2Cl_2 (9 mL) were reacted (1h, r.t., then 1h, r.t.) and subjected to work-up according to method C to give **13b** (111 mg, 82%) as a slowly solidifying colourless oil. KBr/cm^{-1} ν_{max} 3429, 2924, 2360, 1668, 1498, 1456, 1252, 1105, 806, 735, 696; δ_H 0.98 (3H, s, CH_3), 5.04 (4H, brs and s, NH_2, OCH_2), 6.75 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (10H, m), 8.07 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 646 (9.8) [MH^+], 645 (7.8) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(N-tert-butoxycarbonyl-L-alanyl-O-benzyl-L-serinylamino)pyrimidine (**14a**): **12c** (123 mg, 0.21 mmol), N-(*tert*-butoxycarbonyl)-*L*-alanine (**16c**, 79 mg, 0.42 mmol), and DCC (86.5 mg, 0.42 mmol) in dry CH_2Cl_2 (10 mL) were reacted (19 h, r.t.) and subjected to work-up according to method B. Column chromatography on silica gel (CH_2Cl_2/EtOAc 3:1–1:1) gave **14a** (79 mg, 50%) as a slowly crystallising colourless oil. KBr/cm^{-1} ν_{max} 3425, 2927, 2360, 1653, 1502, 1454, 1369, 1245, 1167, 1105, 735, 698; δ_H 1.00 (3H, s, CH_3), 1.38 (3H, d, ^3J = 6.8 Hz, CH_3[Ala]), 1.44 (9H, s, Bu^t), 5.05 (2H, s, OCH_2), 6.76 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.27–7.45 (10H, m), 8.40 (1H, s); δ_C 17.1, 18.7, 26.1, 27.4, 27.5, 28.3, 29.6, 32.8, 37.5, 44.3, 46.5, 53.3, 54.9, 69.2, 70.0, 73.6, 112.4, 114.9, 126.2, 127.5, 127.9, 128.5, 128.6, 129.4, 132.4, 137.2, 137.3, 137.7, 152.6, 155.5, 156.9, 172.7, 183.4; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 761 (2.1) [MH^+], 760 (3.4) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(L-alanyl-O-benzyl-L-serinylamino)pyrimidine (**14b**): **14a** (79 mg, 0.10 mmol) and trifluoroacetic acid (2 × [0.1 mL, 1.3 mmol]) in dry CH_2Cl_2 (5 mL) were reacted (1h, r.t., then 1h, r.t.) and subjected to work-up according to method C to give **14b** (60.8 mg, 89%) as a slowly solidifying colourless oil. KBr/cm^{-1} ν_{max} 3430, 2926, 2360, 1668, 1500, 1456, 1255, 1105, 809, 732; δ_H 0.98 (3H, s, CH_3), 5.05 (2H, s, OCH_2), 6.76 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.22 (1H, d, ^3J = 8.6 Hz), 7.30–7.45 (10H, m), 8.40 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 660 (7.2) [MH^+], 659 (6.5) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(N-tert-butoxycarbonyl-L-alanyl-S-benzyl-L-cysteinylamino)pyrimidine (**15a**): **12e** (94 mg, 0.16 mmol), *N-tert*-butoxycarbonyl)-*L*-alanine (59 mg, 0.31 mmol) and DCC (64 mg, 0.31 mmol) in dry CH_2Cl_2 (10 mL) were reacted (18 h, r.t.) and subjected to work-up according to method B. Column chromatography on silica gel (CH_2Cl_2/EtOAc 3:1–1:1) gave **15a** (67 mg, 56%) as a colourless solid, m.p. 134–136 °C. KBr/cm^{-1} ν_{max} 3423, 2925, 2852, 2360, 1703, 1651, 1506, 1456, 1419, 1373, 1246, 1167, 1024, 696, 418; d_H 1.00 (3H, s, CH_3), 1.37 (3H, d, ^3J = 6.8 Hz, CH_3[Ala]), 1.45 (9H, s, Bu^t), 3.78 (2H, s, SCH_2), 5.05 (2H, s, OCH_2), 6.74 (1H, d, ^4J = 2.3 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.3 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.28–7.45 (10H, m), 8.39 (1H, s); δ_C 17.1, 18.4, 24.9, 25.6, 26.1, 27.4, 27.5, 28.3, 29.6, 32.7, 33.5, 34.0, 36.7, 37.5, 44.3, 46.5, 49.2, 50.3, 52.7, 54.9, 70.0, 112.4, 114.9, 126.2, 127.2, 127.5, 127.9, 128.6, 129.1, 129.5, 132.4, 137.2, 137.7, 138.0, 152.6, 155.4, 156.9, 172.8, 183.6; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 777 (2.9) [MH^+], 776 (3.8) [M^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(L-alanyl-S-benzyl-L-cysteinylamino)pyrimidine (**15b**): **15a** (62 mg, 0.08 mmol) and trifluoroacetic acid [2 × (0.1 mL, 1.3 mmol) in dry CH_2Cl_2 (5 mL) were reacted (3 h, r.t., then 3 h, r.t.) and subjected to work-up according to method C to give **15b** (51.6 mg, 96%) as a colourless solid; m.p. 160–162 °C (dec.). (Found: MH^+, 676.3325. $C_{40}H_{46}O_3N_5S$ requires MH^+, 676.3321). KBr/cm^{-1} ν_{max} 3329, 2927, 2852, 1668, 1626, 1574, 1498, 1454, 1419, 1313, 1244, 1103, 1024, 806, 698; δ_H 0.98 (3H, s, CH_3), 5.04 (2H, s, OCH_2), 6.75 (1H, d, ^4J = 2.7 Hz), 6.78 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.29–7.45 (10H, m), 8.07 (1H, s); δ_C 15.3, 17.1, 20.0, 24.9, 25.6, 26.2, 27.2, 27.4, 27.5, 29.6, 29.7, 32.8, 33.9, 35.3, 36.7, 37.5, 44.3, 46.2, 46.4, 49.2, 50.9, 53.9, 54.8, 65.9, 70.0, 112.4, 114.9, 123.3, 126.2, 127.5, 127.9, 128.6, 128.8, 128.9, 129.1, 132.6, 137.3, 137.4, 137.7, 137.8, 152.6, 156.9, 162.2, 166.8, 168.3, 183.3; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 676 (33.8) [MH^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(O-benzyl-N-tert-butoxycarbonyl-L-serinyl-L-glycyl-S-benzyl-L-cysteinyl amino)pyrimidine (**18a**): A solution of **16** (60 mg, 0.09 mmol) and (N-tert-butoxycarbonyl-O-benzyl-L-serylsuccinimide (**17**, 53 mg, 0.14 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 23 h at r.t. The solution was concentrated in vacuo and the residue was subjected to column chromatography on silica gel (CH_2Cl_2/EtOAc 1:1) to give **18a** (48 mg, 56%) as a slowly crystallising colourless oil. (Found: MH^+, 939.4484. $C_{54}H_{63}O_7N_6S$ requires MH^+, 939.4479). KBr/cm^{-1} ν_{max} 3430, 2930, 1668, 807; δ_H 0.90 (3H, s, CH_3), 1.34 (9H, s, Bu^t), 5.00 (2H, s, OCH_2), 6.67 (1H, d, ^{4}J = 2.3 Hz), 6.70 (1H, dd, ^{3}J = 8.6 Hz, ^{4}J = 2.3 Hz), 7.12 (1H, d, ^{3}J = 8.6 Hz), 7.18–7.37 (15H, m), 8.30 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) m/z (%) 939 (51.3) [MH^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(O-benzyl-L-serinyl-L-glycyl-S-benzyl-L-cysteinylamino)pyrimidine (**18b**): Trifluoroacetic acid (0.06 mL, 0.78 mmol) was added to a solution of **18a** (43 mg, 0.046 mmol) in dry CH_2Cl_2 (3 mL) and the resulting reaction mixture was stirred for 1 h at r.t. Then, further trifluoroacetic acid (0.06 mL, 0.78 mmol) was added to the solution, which was stirred for another hour at r.t. CH_2Cl_2 (30 mL) was added, and the solution was washed with aq. $NaHCO_3$ (20 mL) and water (2 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **18b** (28.7 mg, 74%) as a slowly solidifying colourless oil. (Found: MH^+, 839.3951. $C_{49}H_{55}O_5N_6S$ requires MH^+, 839.3955). KBr/cm^{-1} ν_{max} 3345, 2928, 2359, 1640, 809, 737; δ_H 0.98 (3H, s, CH_3), 5.04 (2H, s, OCH_2), 6.75 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.29–7.45 (10H, m), 8.36 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 839 (3.6) [MH^+].

3-Benzyloxy-estra-1,3,5(10),16-tetraeno[17,16-e]-2'-(O-benzyl-L-serinyl-L-glycyl-O-benzyl-L-serinylamino)pyrimidine (**19**): A solution

of **13b** (64 mg, 0.10 mmol) and *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-*L*-serylsuccinimide (**17**, 58 mg, 0.15 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 17 h at r.t. The solution was concentrated *in vacuo* and the residue was subjected to column chromatography on silica gel (CH_2Cl_2/EtOAc 1:1 – 1:2) to give BOC-protected **19** (45.8 mg, 50%). BOC-protected **19** (45.8 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and trifluoroacetic acid (0.06 mL, 0.78 mmol) was added. The resultant solution was stirred for 1 h at r.t. Then, additional trifluoroacetic acid (0.06 mL, 0.78 mmol) was added and the solution stirred for 2 h at r.t. CH_2Cl_2 (30 mL) was added to the solution, which was washed with aq. $NaHCO_3$ (20 mL) and water (2 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **19** (35 mg, 86%) as a slowly solidifying oil. KBr/cm^{-1} ν_{max} 3341, 2926, 2360, 1636, 1508, 1455, 1418, 1241, 1103, 1027, 809, 737, 697; δ_H 0.92 (3H, s, CH_3), 4.98 (2H, s, OCH_2), 6.68 (1H, d, ^4J = 2.6 Hz), 6.71 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.14 (1H, d, ^3J = 8.6 Hz), 7.20–7.38 (10H, m), 8.00 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 823 (65.7) [MH^+].

3-Benzyloxyestra-1,3,5(10),16-tetraeno[17,16-e]-2′-(L-serinyl-L-alanyl-O-benzyl-L-serinylamino)pyrimidine (**20**): DCC (38 mg, 0.18 mmol), was added to a solution of **14b** (61 mg, 0.09 mmol) and *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-*L*-serine (**17**, 54 mg, 0.18 mmol) in dry CH_2Cl_2 (7 mL) and the resulting reaction was stirred for 5 h at r.t. The precipitated urea was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/EtOAc 2:1-1:1) to give the *BOC*-protected Ala-Ala-Cys-aminopyrimidinoestrane (31 mg, 36%). The compound was taken up in CH_2Cl_2 (3 mL), trifluoroacetic acid (0.05 mL, 0.65 mmol) was added to the solution, and the mixture was stirred for 1 h at r.t. Then, further trifluoroacetic acid (0.05 mL, 0.65 mmol) was added to the solution, which was stirred for an additional hour at r.t. CH_2Cl_2 (30 mL) was added to the mixture, which was washed with aq. $NaHCO_3$ (20 mL) and water (2 × 20 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **20** (24 mg, 91%) as a slowly crystallising solid. (Found: MH^+, 837.4335. $C_{50}H_{57}O_6N_6$ requires MH^+, 837.4340). KBr/cm^{-1} ν_{max} 3337, 2927, 2856, 2360, 1636, 1604, 1499, 1454, 1419, 1375, 1240, 1103, 1027, 809, 735, 697; δ_H 0.98 (3H, s, CH_3), 1.39 (3H, d, ^3J = 6.8 Hz, Me[Ala]), 5.05 (2H, s, OCH_2), 6.75 (1H, d, ^4J = 2.7 Hz), 6.79 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.7 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.29–7.45 (10H, m), 8.40 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 837 (8.2) [MH^+].

3-Benzyloxyestra-1,3,5(10),16-tetraeno[17,16-e]-2′-(L-alanyl-L-alanyl-S-benzyl-L-cystinylamino)pyrimidine (**21**): DCC (32 mg, 0.15 mmol), was added to a solution of **15b** (52 mg, 0.077 mmol) and *N*-(*tert*-butoxycarbonyl)-*L*-alanine (29 mg, 0.15 mmol) in dry CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 12 h at r.t. The precipitated urea was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/EtOAc 1:1-1:2) to give the *BOC*-protected Ala–Ala–(Bn–Cys)–aminopyrimidinoestrane (27 mg, 42%). It was taken up in dry CH_2Cl_2 (3 mL), trifluoroacetic acid (0.05 mL, 0.65 mmol) was added, and the reaction mixture was stirred for 1 h at r.t. CH_2Cl_2 (30 mL) was added to the solution, which was washed subsequently with aq. $NaHCO_3$ (20 mL) and water (2 × 20 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **21** (22.8 mg, 95%) as a slowly solidifying solid. KBr/cm^{-1} ν_{max} 3329, 2927, 2853, 2359, 1648, 1605, 1560, 1498, 1455, 1424, 1377, 1258, 1105, 1027, 808, 736, 696; δ_H 0.98 (3H, s, CH_3), 5.04 (2H, s, OCH_2), 6.75 (1H, d, ^4J = 2.6 Hz), 6.78 (1H, dd, ^3J = 8.6 Hz, ^4J = 2.6 Hz), 7.21 (1H, d, ^3J = 8.6 Hz), 7.29–7.45 (10H, m), 8.07 (1H, s); MS (FAB, 3-nitrobenzyl alcohol) *m/z* (%) 747 (48.3) [MH^+].

Received 5 February 2009; accepted 14 April 2009
Paper 09/0435 doi: 10.3184/030823409X460722
Published online: 30 June 2009

References

1 C. Gunanathan, A. Pais, E. Furman-Haran, D. Seger, E. Eyal, S. Mukhopadhyay, Y. Ben-David, G. Leitus, H. Cohen, A. Vilan, H. Degani and D. Milstein, *Bioconjug. Chem.*, 2007, **18**, 1361.
2 B. Ferber, S. Top, R. Welter and G. Jaouen, *Chem. Eur. J.*, 2006, **12**, 2081.
3 B. Ferber, S. Top, A. Vessieres, R. Weller and G. Jaouen, *Organometallics*, 2006, **25**, 5730.
4 D.Y. Chi, J.P. O'Neil, C.J. Anderson, M.J. Welch and J.A. Katzenellenbogen, *J. Med. Chem.*, 1994, **37**, 928.
5 F. Wuest, K.E. Carlson, J.A. Katzenellenbogen, H. Spies and B. Johannsen, *Steroids*, 1998, **63**, 665.
6 J.B. Arterburn, K.V. Rao and M.C. Perry, *Angew. Chem. Int. Ed. Engl.*, 2000, **39**, 771.
7 P. Wolohan and D.E. Reichert, *Steroids*, 2007, **72**, 247.
8 M.C. Melo e Silva, L. Patrício, L. Gano, M.L. Sa e Melo, E. Inohae, S. Mataka and T. Thiemann, *Appl. Rad. Isotopes*, 2001, **54**, 227.
9 M. das Neves Oliveira, M. Videira, A. Almeida, L. Gano, M. Watanabe, T. Thiemann, A.C. Santos, M. Botelho and C. Oliveira, *J. Labell. Compd. Radiopharm.*, 2006, **49**, 559.
10 C. Neto, M.C. Oliveira, L. Gano, F. Marques, I. Santos, G. Morais, T. Yasuda, T. Thiemann, C. Santos, F. Botelho and C. Oliveira, *Appl. Radiat. Isotopes*, 2009, **67**, 301.
11 C. Neto, M.C. Oliveira, L. Gano, F. Marques, I. Santos, G. Morais, T. Yasuda, T. Thiemann, F. Botelho and C.F. Oliveira, *Curr. Radiopharmaceut.*, 2009, **2**, 83.
12 T. Matsumoto, M. Watanabe, S. Mataka and T. Thiemann, *Steroids*, 2003, **68**, 751.
13 J. Lister-James, B.R. Moyer and T. Dean, *J. Nucl. Med.*, 1996, **40**, 221.
14 S. Liu and D.S. Edwards, *Chem. Rev.*, 1999, **99**, 2235.
15 S.M. Okarvi, P. Adriaens and A.M. Verbruggen, *J. Labell. Compd. Radiopharm.*, 1997, **10**, 853.
16 A.M. Verbruggen, *Dev. Nucl. Med.*, 1996, 31.
17 P. Tapolcsanyi, J. Woelfling, G. Falkay, A. Marki, R. Minorics and G. Schneider, *Steroids*, 2002, **67**, 371.
18 H. Brederick, R. Sell and F. Effenberger, *Chem. Ber.*, 1964, **97**, 3407.
19 T. Matsumoto, T. Matsumoto, M. Watanabe, S. Mataka and T. Thiemann, *Acta Cryst., Sect. C*, 2004, **C60**, o501.
20 H. Khorana, *Chem. Rev.,* 1953, **53**, 145.
21 W.C. Shieh, J.A. Carlson and M.E. Shore, *Tetrahedron Lett.*, 1999, **44**, 7167.
22 T. Mukaiyama, R. Matsueda and M. Suzuki, *Tetrahedron Lett.*, 1970, **22**, 1901.
23 R.A.W. Johnstone and M.E. Rose, *Tetrahedron*, 1979, **35**, 2169.
24 J. Hart, W.P. Hong and L.Y. Hsu, *J. Org. Chem.*, 1987, **52**, 4665.