Supporting Information

Complex Polyfluoride Additives in Fmoc-Amino Acid and Peptide Acid Fluoride Coupling Processes. Enhanced Reactivity and Avoidance of Stereomutation.

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Benzyltriphenylphosphonium dihydrogentriflouride (PTF). A solution of 7.8 g (20 mmol) of benzyltriphenylphosphonium chloride in 100 ml of CH_2Cl_2 was stirred vigorously with a solution of 78.2 g (1 mole) of KHF_2 in 200 ml of distilled water for 0.5 h in a plastic flask. After separation of layers the organic layer was stirred two more times as indicated above with fresh 200-ml portions of KHF_2 solution. Finally, the organic layer was separated and the solvent removed in vacuum. The solid residue upon recrystallization from methanol-ether gave a colorless crystalline mass (8.16 g, yield 99%), mp 148-150C; ^{1}H NMR ($CDCl_3$): δ 4.85 (d, 2, CH_2); 7.0-7.7 (m, 22); Anal. Calcd for $C_{25}H_{24}PF_3$: C, 72.80; H, 5.86; F, 13.82. Found: C, 72.95; H, 5.80; F, 13.60.

Manual Solid Phase Syntheses. All syntheses were carried out in 5- or 10-ml plastic syringes using a PAL-PEG-PS resin with 4 eqs. excess amino acid, 4 eqs. of additive, if used, deblocking times 7 min and coupling times 30 min. Preactivation via DCC or DIC was carried out in some cases in CH_2Cl_2 followed by evaporation of solvent and transfer to DMF for coupling. In other cases DMF was used for both activation and coupling. Parallel runs with and without the fluoride additive were carried out side-by-side. Specific details are given on the attached HPLC curves. IR determinations were carried out in CH_2Cl_2 (NaCl cells) or DMF (CaF_2)

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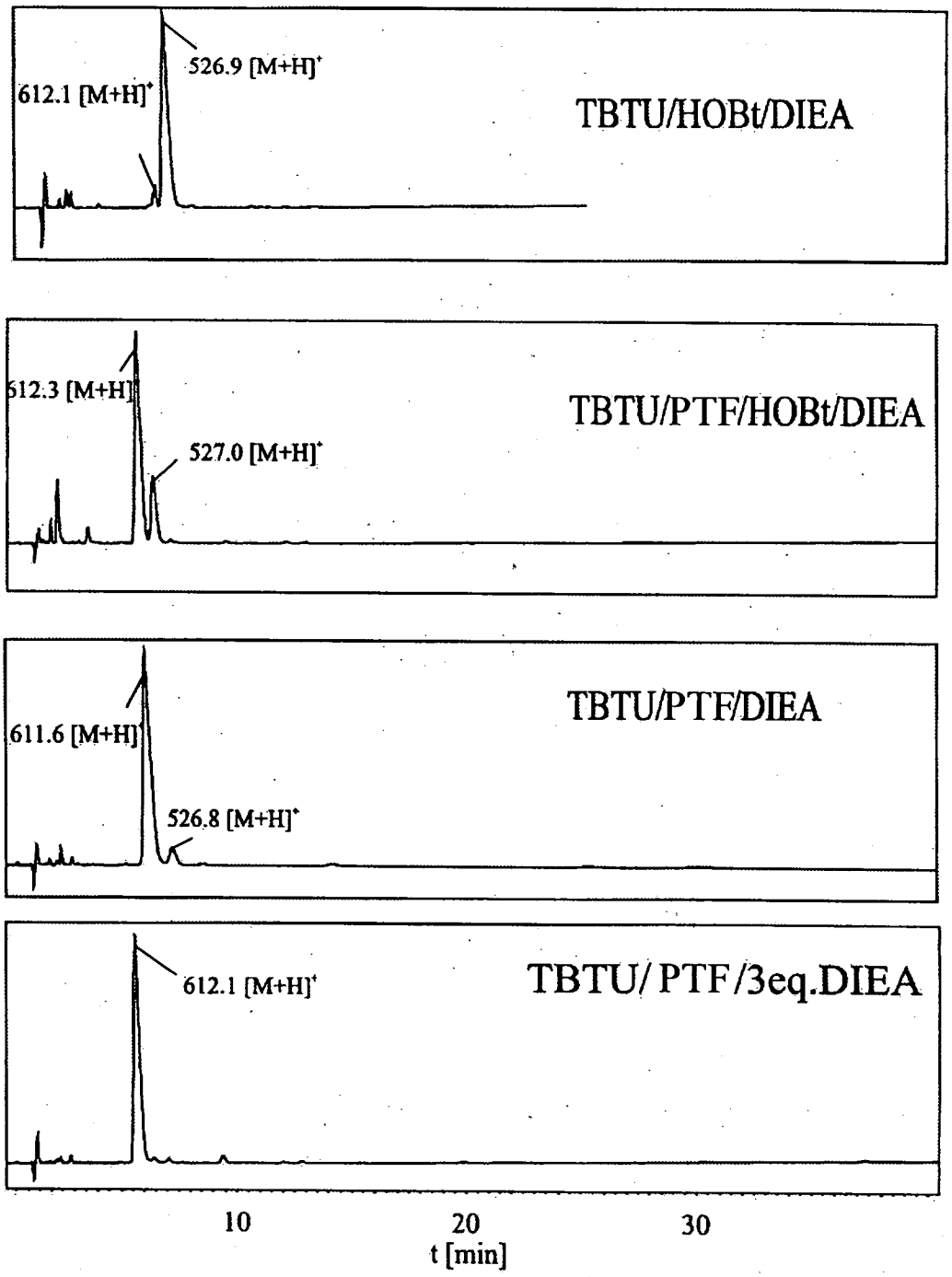
For non-carbodiimide coupling reagents, *e.g.*, TFFH, HATU and TBTU, parallel runs were made similarly in the absence and presence of additive. Co-injection experiments confirmed that the presence of additive always guaranteed the best results. See Figs. 2-5.

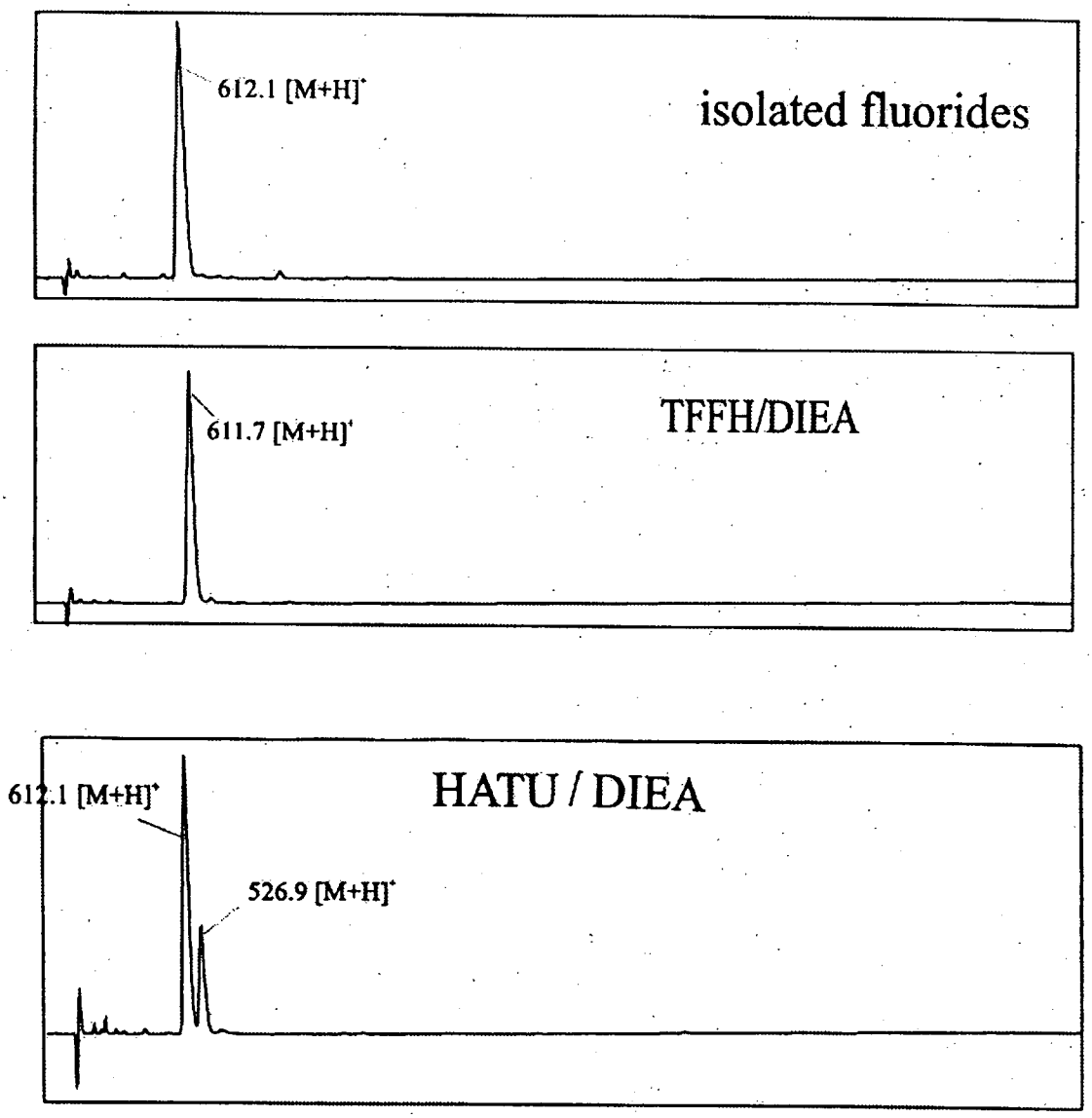
Automated Solid Phase Syntheses of H-Tyr-Aib-Aib-Phe-Leu-NH_2 in the Presence or Absence of PTF. An ABI 433A synthesizer was used with a preactivation time of 20 min and double coupling (15 min each). The resin was TG-SRAM (5.0 g) with a loading of 0.24 mmol/g. The excess of Fmoc amino acid was 8.3 eq., conc. 0.33 *M* in DMF with 1-3 eq. DIEA (related to the eqs. of amino acid) and 7 min deblocking via 20% piperidine in DMF. The actual HPLC spectra are reproduced below with the identiy of the two product peptides, the desired pentapeptide and the des-Aib-tetrapeptide identified by LC/MS according to the molecular weights 610.7 and 525.7, respectively.

Fig. 1. **Automated Solid Phase Syntheses of H-Tyr-Aib-Aib-Leu-NH_2 in the Presence or Absence of PTF.**



Tyr-Aib-Aib-Phe-Leu-NH_2: 610.7 g/mol
Tyr-Aib-Phe-Leu-NH_2:525.65 g/mol

Fig. 1 (continued)



Tyr-Aib-Aib-Phe-Leu-NH_2: 610.7 g/mol
Tyr-Aib-Phe-Leu-NH_2:525.65 g/mol

Manual Solid Phase Syntheses of H-Tyr-Aib-Aib-Phe-Leu-NH_2. All syntheses were carried out in 5- or 10-ml plastic syringes using a PAL-PEG-PS resin using a 5-fold excess of amino acid, deblocking times of 7 min and coupling times of 30 min. Preactivation via DCC or DIC was carried out in some cases in DCM, followed by evaporation of solvent and transfer to DMF for coupling. In other cases, DMF was used for both activation and coupling. Parallel runs with and without additive were carried out side-by-side. For non-carbodiimide coupling reagents, e.g. TFFH, HATU and HBTU, parallel runs were made similarly in the presence and absence of additive. Co-injection experiments (HPLC) with authentic samples of the pentapeptide and the des-Aib-tetrapeptide confirmed that the presence of the additive always guaranteed the best result. For the results see Table 1.

Table 1

Manual Solid Phase Assembly of H-Tyr-Aib-Aib-Phe-Leu-NH_2[a]

Solvent	Coupling Reagent	Base[b]	Preactivation time (min)	Additive	Yield %	Des-Aib-peptide (%)[c]
DMF	DIC	--	15	--	32.2	78.1
DMF	DIC	--	15	$(C_6H_5)_4P^+H_2F_3^-$	69.7	18.8
DMF	DIC	--	15	nBu$_4N^+(C_6H_5)_3SnF_2^-$	40.8	49.1
DCM-DMF[d]	DCC	--	7	--	90.3	73.5
DCM-DMF[d]	DCC	--	7	$(C_6H_5)_4P^+H_2F_3^-$	97.8	10.5
DMF	TFFH	DIEA (2)	7	--	87.1	9.0
DMF	HATU	DIEA (2)	7	--	83.4	4.5

[a] Manual syntheses, 5-fold excess of amino acid, coupling time, 30 min.
[b] The number of equivalents is given in parentheses.
[c] The product consists of a mixture of penta- and tetrapeptide. The extent of the undesired tetrapeptide is given here.
[d] In these cases preactivation is carried out in DCM, the solvent removed, and coupling carried out in DMF.

Table 2

Loading of Fmoc-Aib-OH onto Preformed Sequence H-Aib-Gln-Gln-Phe-TG-SAC[a]

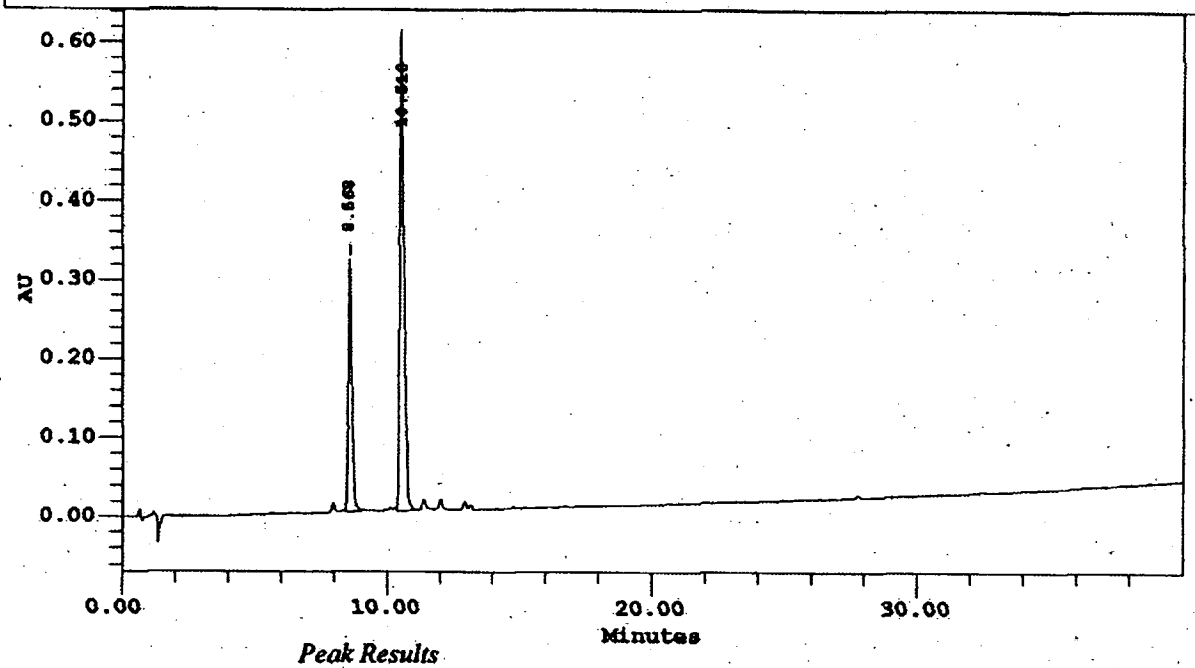
Coupling Procedure	Target Peptide (%)[b]	des-Aib peptide (%)[b]
2 equiv. TFFH	69.7	30.3
2 equiv. TFFH/PTF	77.1	22.9
5 equiv.TFFH	89.7	10.3
5 equiv. TFFH/PTF	97.2	2.8

[a] Preactivation for 20 min. with 2 equiv. DIEA per equiv. of amino acid, concentration 0.3 M, single coupling for 30 min.
[b] These percentages refer to the ratio between the penta- and tetrapeptides. The HPLC traces show other unidentified peaks. See traces below.

Table 2 (continued)

Project Name:	ANALYTISCH	Channel ID:	17850
Report Method:	Polyencap_5_95B	Result ID:	17856
Processing Method:	Analytisch	Date Acquired:	07/14/97 01:06:09 PM
Acq Meth Set:	Grad_5_95B	Date Processed:	07/14/97 02:05:36 PM
Channel:	996		
	220nm		

Sample Name:	2 eq TFFH	Column :	Polyencap
Vial:	77	Gradient :	5-95%B 40 min
Injection:	1	A :	0.1% TFA
Volume:	20.00	B:	80% ACN / 20%H20 / 0.1% TFA



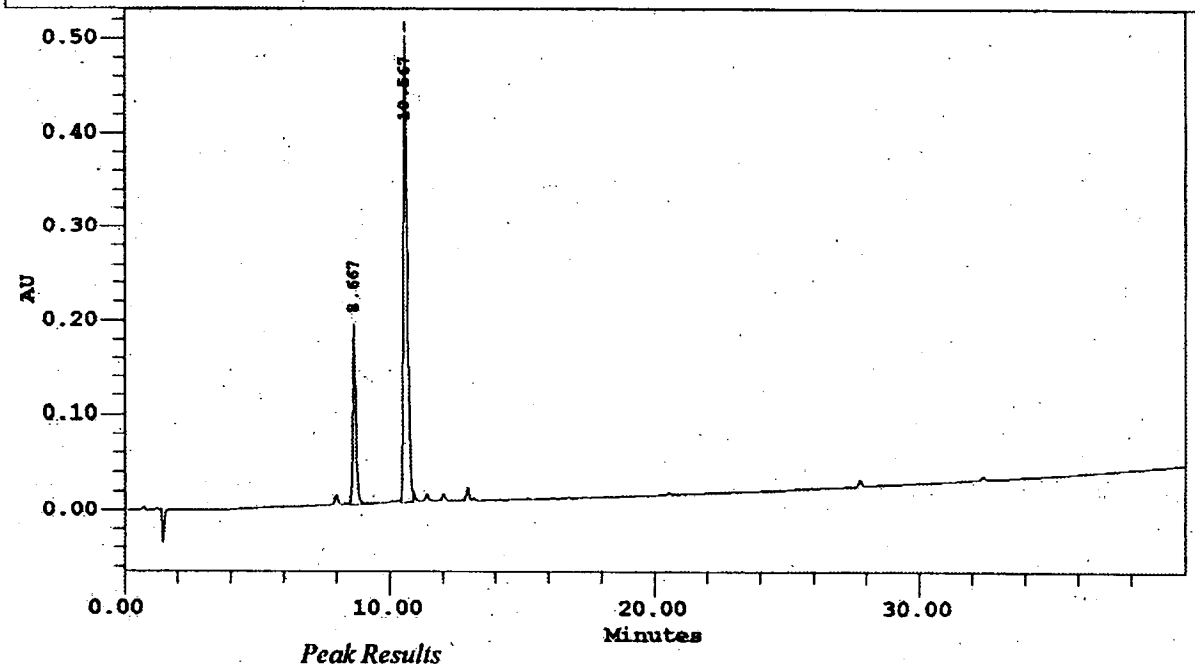
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	% Area	Height (uV)
1		8.57	2952422	30.29	320100
2		10.52	6793183	69.71	602004

Table 2 (continued)

Project Name:	ANALYTISCH	Channel ID:	17847
Report Method:	Polyencap_5_95B	Result ID:	17852
Processing Method:	Analytisch	Date Acquired:	07/14/97 12:03:40 PM
Acq Meth Set:	Grad_5_95B	Date Processed:	07/14/97 01:10:17 PM
Channel:	996 220nm		

Sample Name:	2 eq TFFH/PTF	Column :	Polyencap
Vial:	76	Gradient :	5-95%B 40 min
Injection:	1	A :	0.1% TFA
Volume:	20.00	B:	80% ACN / 20%H20 / 0.1% TFA



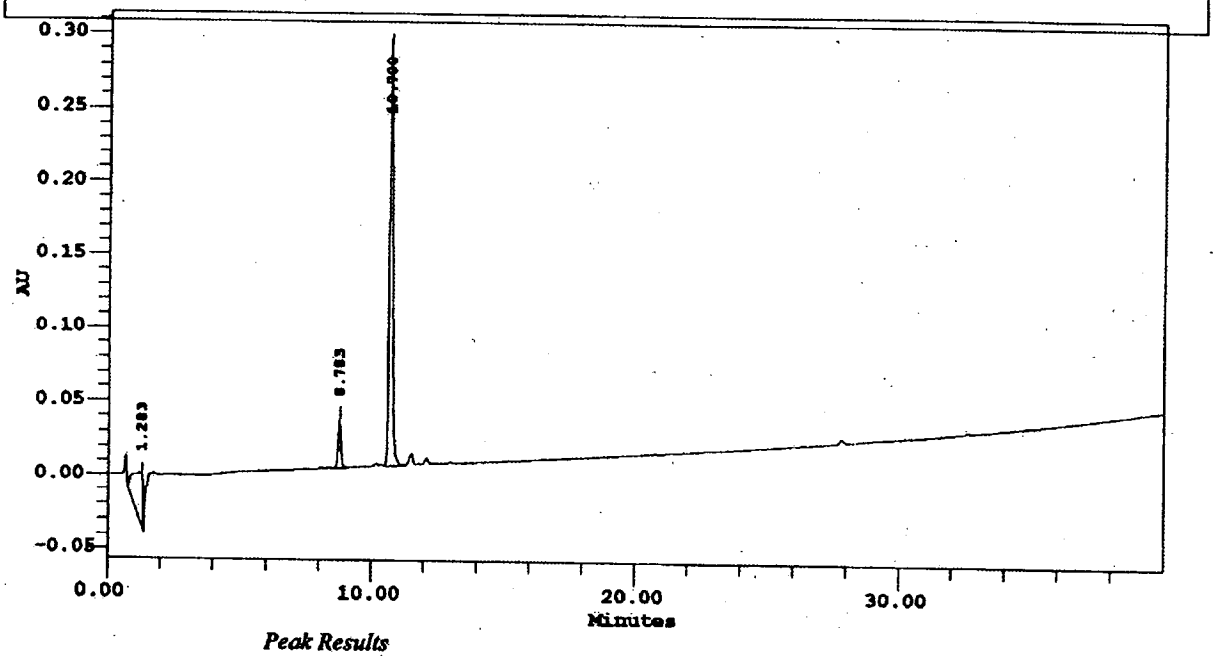
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	% Area	Height (uV)
1		8.67	1493023	22.91	180212
2		10.57	5022559	77.09	494952

Table 2 (continued)

Project Name:	ANALYTISCH	Channel ID:	17670
Report Method:	WatersC18_5_95B	Result ID:	17684
Processing Method:	Analytisch	Date Acquired:	07/08/97 12:19:54 PM
Acq Meth Set:	Grad_5_95B	Date Processed:	07/08/97 01:52:14 PM
Channel:	996 220nm		

Sample Name:	5eq TFFH	Column :	Waters C18
Vial:	2	Gradient :	5-95%B 40 min
Injection:	1	A :	0.1% TFA
Volume:	20.00	B:	80% ACN/20%H2O 0.1%TFA



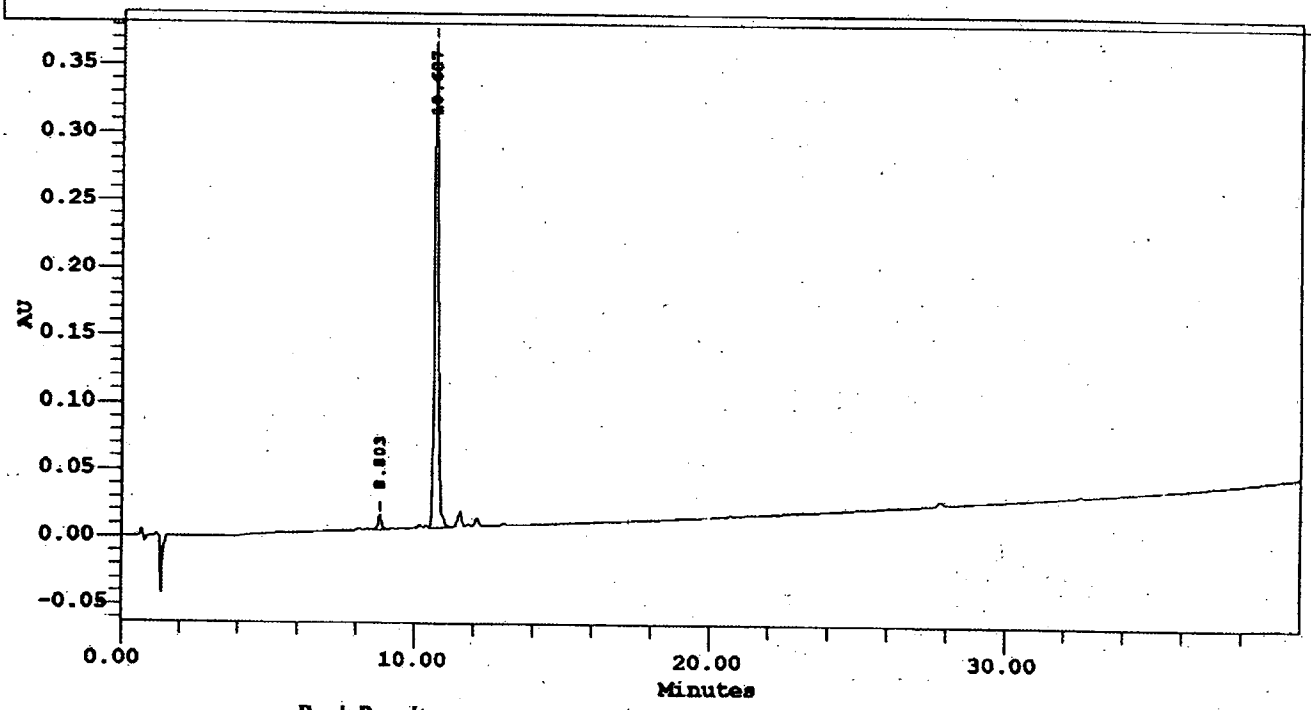
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	% Area	Height (uV)	
1		1.28	792701	22.66	36168	
2		8.78	244111	6.98	33678	→ 10,3%
3		10.70	2460952	70.36	293680	→ 89,7%

Table 2 (continued)

Project Name:	ANALYTISCH	Channel ID:	17673
Report Method:	WatersC18_5_95B	Result ID:	17701
Processing Method:	Analytisch	Date Acquired:	07/08/97 01:25:35 PM
Acq Meth Set:	Grad_5_95B	Date Processed:	07/08/97 02:26:57 PM
Channel:	996		
	220nm		

Sample Name:	5eq TFFH/UFA	Column :	Waters C18
Vial:	3	Gradient :	5-95%B 40 min
Injection:	1	A :	0.1% TFA
Volume:	20.00	B:	80% ACN/20%H2O 0.1%TFA

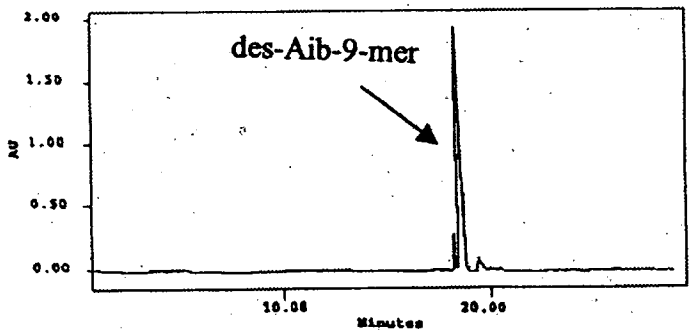


Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	% Area	Height (uV)
1		8.80	93273	2.79	11743
2		10.69	3254982	97.21	360941

Fig. 2: **Manual Synthesis of H-Val-Gln-Aib-Aib-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2($Aib^{67}Aib^{68}$-ACP)**

a) without additive DIC/8 equiv. AA, 30-min. coupling



(b) with PFT additive DIC/4 equiv. AA, 4 equiv of PTF, 30-min coupling

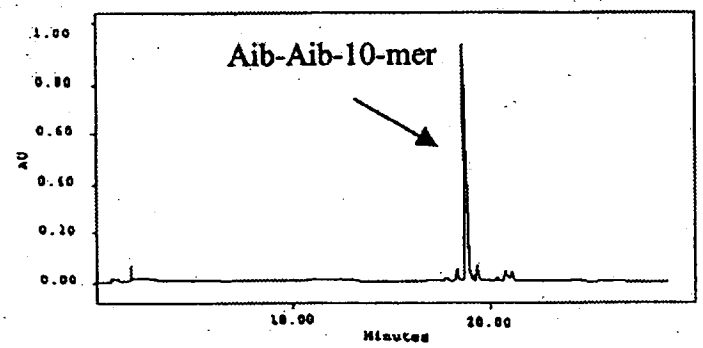
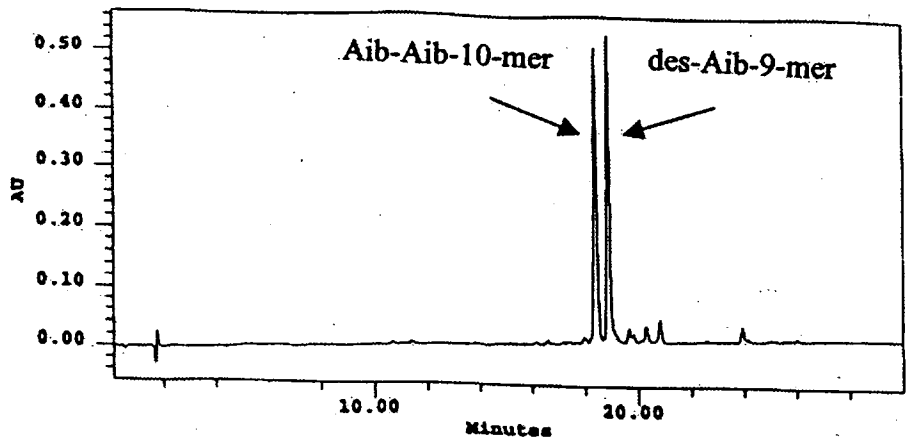


Fig. 3: **Manual Synthesis of H-Val-Gln-Aib-Aib-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2($Aib^{67}Aib^{68}$-ACP)**

(a) without additive HATU/4 equiv. AA, 8 equiv. DIEA, 30-min coupling



(b) with PFT additive HATU/4equiv. of PTF, 8 equiv. DIEA, 30-min. coupling

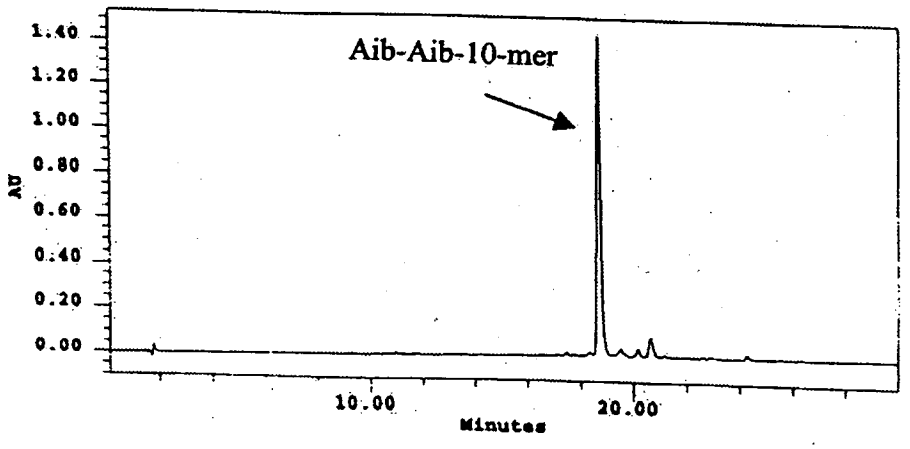
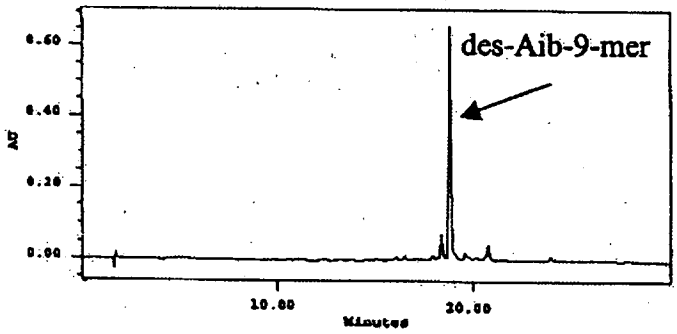


Fig 4: **Manual Synthesis of H-Val-Gln-Aib-Aib-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2($Aib^{67}Aib^{68}$-ACP)**

(a) without additive TBTU/4 equiv. AA, 8 equiv. DIEA, 30-min. coupling



(b) with PFT additive TBTU/4 equiv. AA, 4 equiv. DIEA, 30-min. coupling

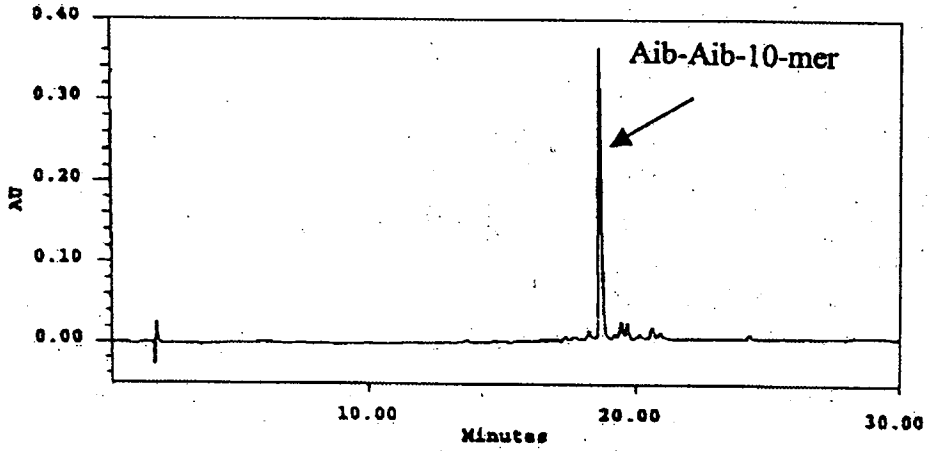
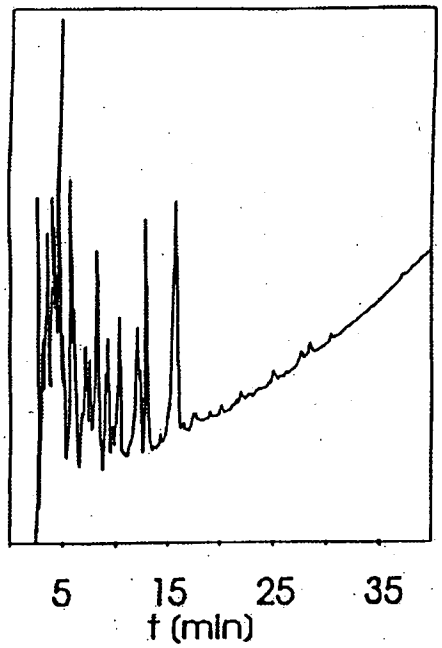
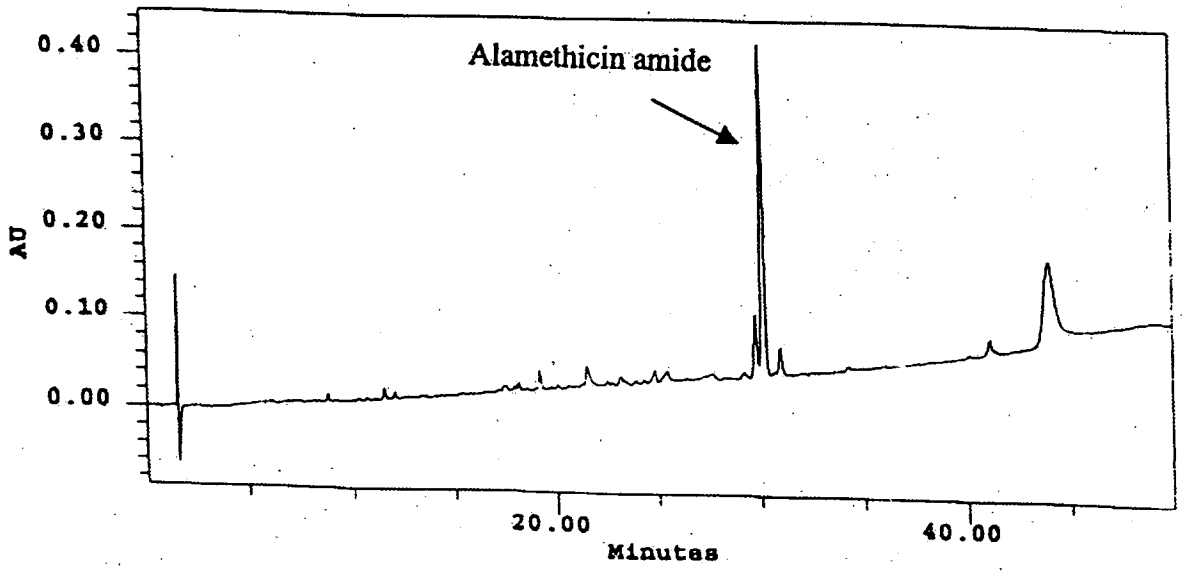


Fig. 5: **Manual Syntheses of Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe-NH_2 (Alamathicin F30 amide)**

(a) without additive. HBTU/4 equiv. AA, 8 equiv. DIEA 30-min. coupling



(b) with PTF additive HBTU/4 equiv. AA, 4 equiv. of PTF, 8 equiv. DIEA 30-min.coupling



Study of the Loss of Configurtion During the Solid Phase Coupling of Fmoc-His(Trt)-OH to H-Pro-PAL-PEG-PS

In a 5-ml syringe tube fitted at the bottom with a Teflon frit was weighed 100 mg of Fmoc-Pro-PAL-PEG-PS (0.2 mmol/g). The resin was washed with DMF (5 x 5 ml), treated with 20% piperidine in DMF (4 ml) for 7 min and then washed with DMF (5 x 5 ml). After removing the solvent by water aspirator vacuum a solution of Fmoc-His(Trt)-OH (3 equiv., 0.06 mmol), additive (3 equiv.), DIEA (6 equiv.) and the coupling reagent (see Table 3) in 0.4 ml of DMF was preactivated for 7 min and added to the resin. During the 30 min. coupling time the resin was stirred with a Teflon stick. At the end of 30 min. the resin was washed with DMF (5 x 5 ml), CH_2Cl_2 (5 x 5 ml), EtOH 1 x 5 ml), ether (2 x 5 ml) and dried *in vacuo* for 0.5 h. The dry resin was treated with 2 ml of 100% trifluoroacetic acid (TFA) for 1 hr. The TFA was removed by filtration into a collection vessel and the resin washed on the filter with CH_2Cl_2 (2 x 5 ml). The combined filtrates were concentrated in vacuo at room temperature to dryness and the residue dissolved in 1 ml of CH_3CN for analysis by direct injection onto an HPLC column. For the results see Table 3 and Fig. 6.

Table 3

System: Fmoc-His(Trt)-OH +H-Pro-PAL-PEG-PS, with or without PTF

CR (amt)	Base (amt)	Additive	Preactiv Time (min)	DL (%)
TFFH (31.7 mg)	DIEA (41.8 µl)	-	7	7.40
TFFH (31.7 mg)	DIEA (41.8 µl)	+	7	1.82
TBTU (38.5 mg)	DIEA (41.8 µl)	-	7	2.79
TBTU (38.5 mg)	DIEA (41.8 µl)	+	7	1.45
HATU (45.6 mg)	DIEA (41.8 µl)	-	7	3.24
HATU (45.6 mg)	DIEA (41.8 µl)	+	7	1.52

Fig. 6: **HPLC Curves for the Separation of LL- and DL-Fmoc-His(Trt)-Pro-NH_2 via Coupling in the Absence and the Presence of PTF.**

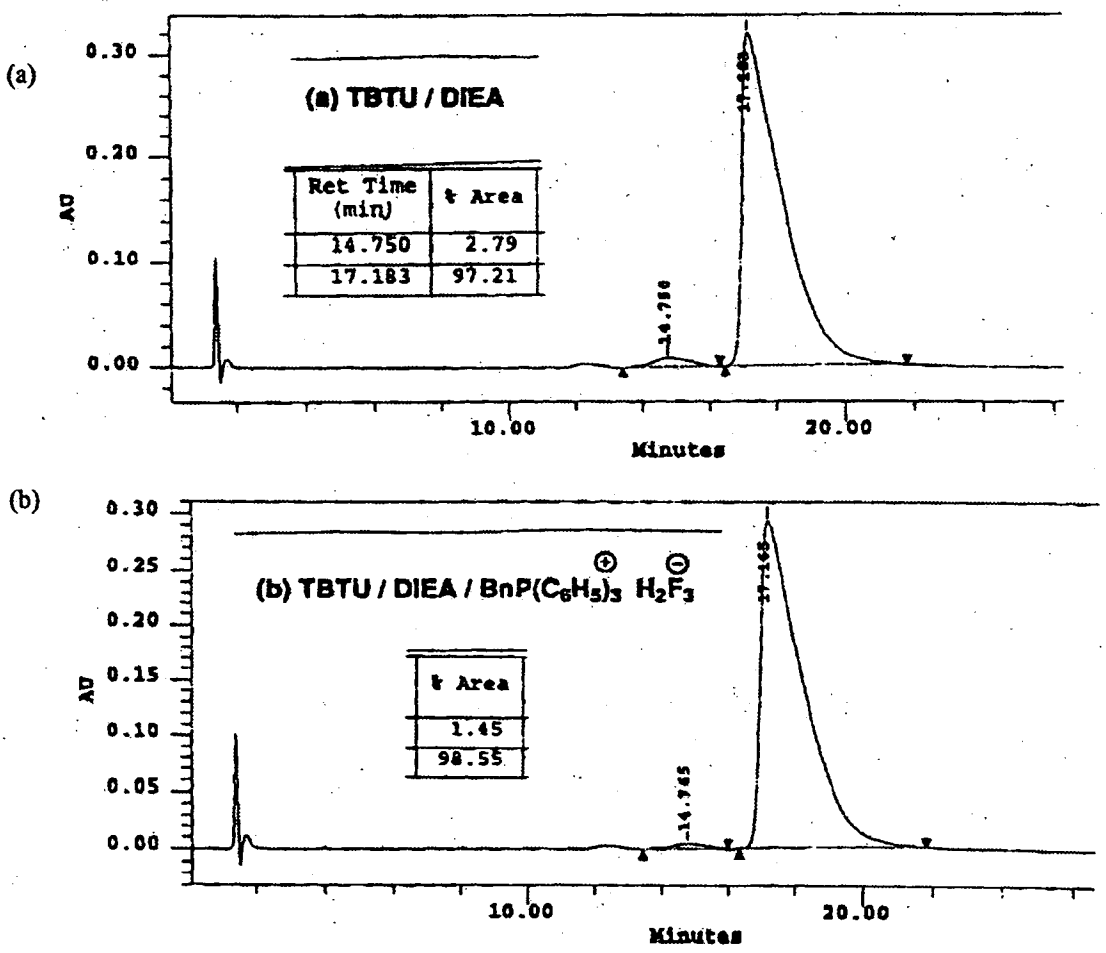
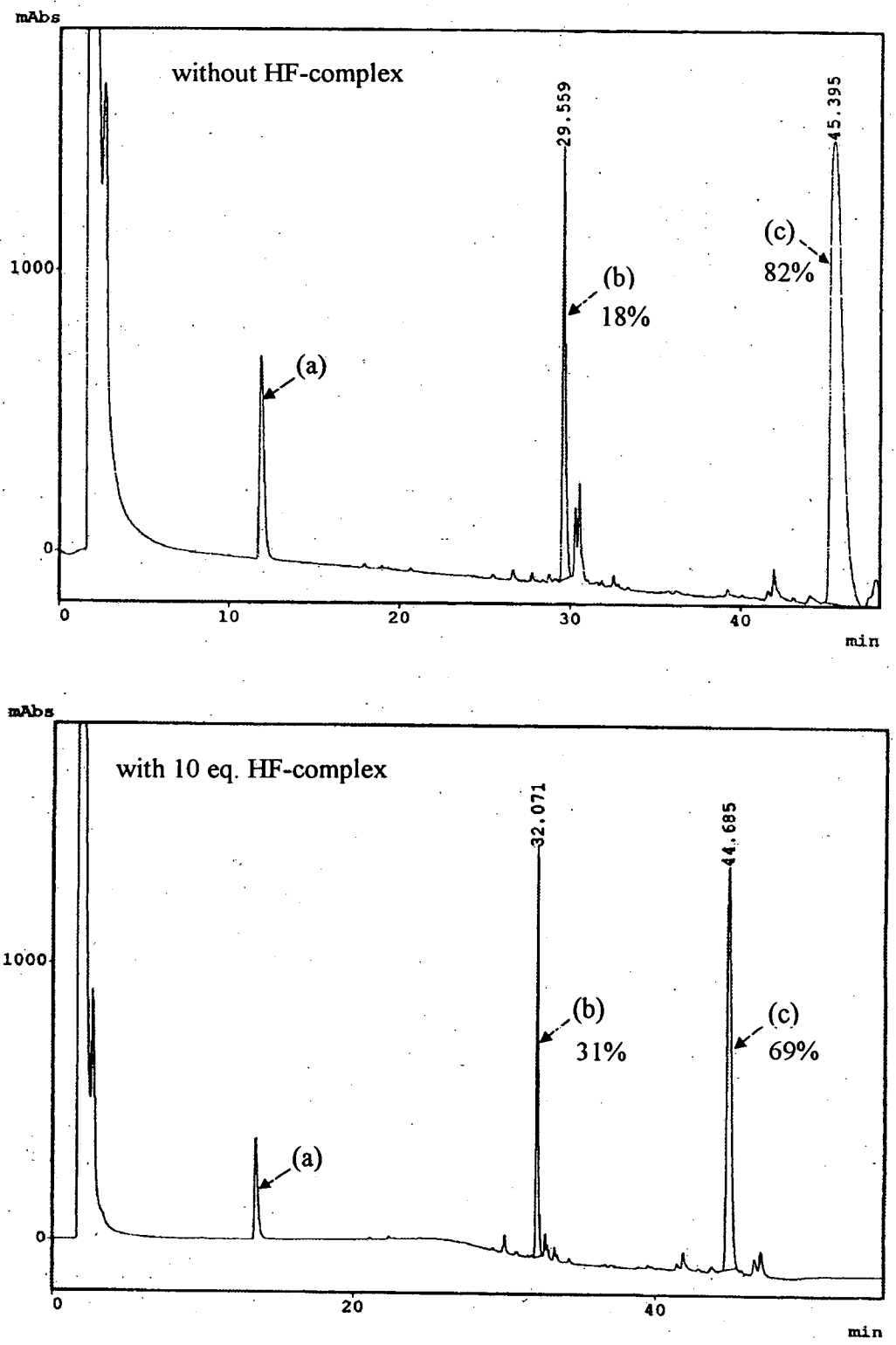


Fig. 7: **Coupling of Fmoc-Val-Gln-(Trt)Ala-Ala-OH with H-Ile-Asp(O-t-Bu)-Tyr(t-Bu)-Ile-Asn-(Trt)-Gly-NH_2 via HAPyU in the Absence and Presence of $Py(HF)_n$.**



(a) Bipyrrolidine urea, (b) Fmoc-Val-Gln(Trt)-Ala-Ala-OH, (c) Fmoc-Val-Gln(Trt)-Ala-Ala-Ile-Asp(OtBu)-Tyr(tBu)-Ile-Asn(Trt)-Gly-NH_2

Fig. 8: **Infrared study of the reaction of Z-Gly-Ala-F with p-nitroaniline (a) oxazolone derived from treatment of Z-Gly-Ala-OH with DIC; (b) Z-Gly-Ala-F derived from oxazolone by treatment with py(HF)$_n$; (c) 1 min after addition of p-nitroaniline**