SYNTHESIS AND *IN VITRO* ACTIVITY OF NEW SEMI-SYNTHETIC COUMERMYCIN ANALOGS: CHEMICAL MODIFICATION AT THE C-3 AMIDE

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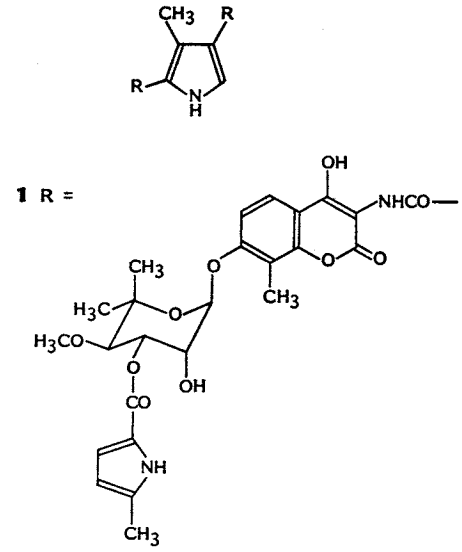
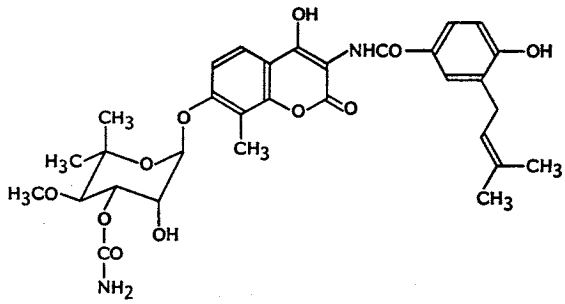
Several new semi-synthetic coumermycin analogs, which carry a polar substituent at the C-3 amide moiety have been prepared. *In vitro* antibacterial activity of these new analogs against Gram-positive organisms, particularly methicillin-resistant strains of Staphylococci species has been described.

Coumermycin A_1 (**1**) is a coumarin-containing antibiotic, discovered nearly two decades ago.[1~3] Coumermycin A_1 and structurally related antibiotics[4] such as novobiocin (**2**) are currently undergoing a renewed interest for two major reasons. One is that this class of antibiotics, particularly **1**, possesses potent antibacterial activity against methicillin-resistant strains of Staphylococci species[5~7] which have become clinically important pathogens in recent years.[7,8] The other reason is their mechanism of action. Unlike other antibiotics such as β-lactams and macrolides, this class of antibiotics is known to act as an inhibitor of bacterial DNA gyrase.[9,10] Although extensive studies on semi-synthetic coumermycin analogs were reported in the past,[11] because of the resurgent interest, we decided to reinvestigate the chemical modification of **1**. Our goal was to search for new analogs having improved pharmaceutical properties, such as good water-solubility and oral absorbability, while retaining the good antibacterial activity particularly against methicillin-resistant strains of Staphylococci species. In spite of its excellent antibacterial activity, **1** is poorly soluble in water which makes parenteral administration unsatisfactory. This poor water-solubility is also believed to be partly responsible for its unfavorable pharmacokinetics and irritation liability.[11] We reasoned that this poor water-solubility was primarily due to its large molecular size, the presence of two coumarin moieties and the lack of strong polar substituents in the molecule. Previous workers have reported[11] a number of semi-synthetic analogs which are monomeric noviosyloxycoumarin derivatives having a variety of carboxy-amido substituents at the 3-position of the coumarin ring such as benzamido derivative **3**[12] and 4-hydroxy-3-(3-methylbutyl)benzamido derivative **4**.[13] However, most of them did not carry a strong polar substituent such as an amino group or a carboxylic acid moiety. Therefore, we have specifically directed our efforts towards the introduction of such a strong polar substituent at the C-3 amide moiety. This paper describes the preparation of new semi-synthetic analogs which contain aminoacetamide moieties **5** and **6**, and aminoethoxybenzamide group **7**. The latter compound **7** was chosen because the 4-hydroxy-3-(3-methylbutyl)benzamide derivative **4** was reported to be one of the most potent analogs.[11] We also describe here the preparation of a carboxy analog **8a** and its carbamoyl derivative **8b**. *In vitro* antibacterial activity of these new analogs and their synthetic intermediates are also





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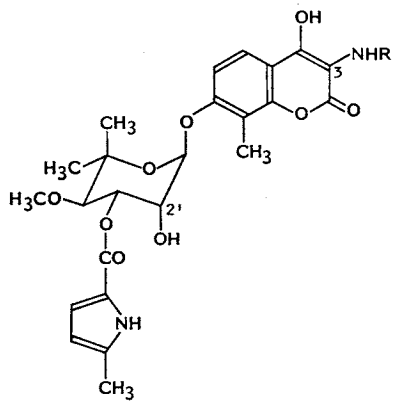

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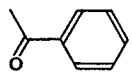

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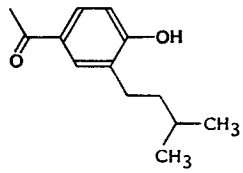

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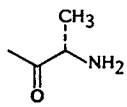
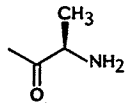
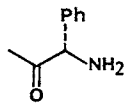


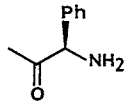
5b R =



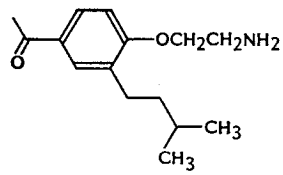
6a R =



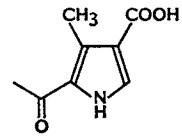
6b R =



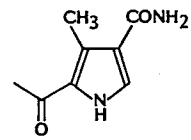
7 R =



8a R =



8b R =

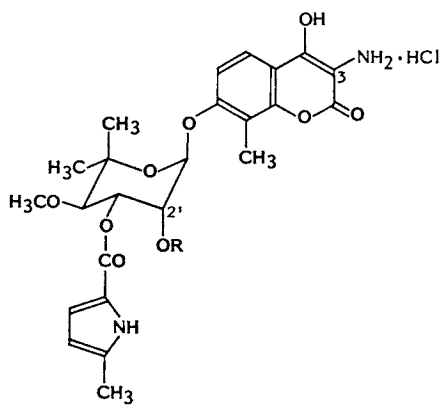


described.

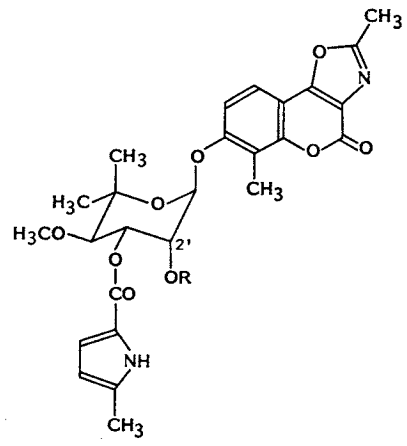
Chemistry

Recently we reported[14] a new process to key intermediates 2′-acetyl PNC-amine† **9** and PNC-amine **10** which involved a selective cleavage of the oxazole moiety in noviosyloxycoumarins **11** and **12**. Using these intermediates, the alanine- and phenylglycine-containing analogs **5** and **6** were prepared by either condensation of 2′-acetyl PNC-amine **9** with *N*-protected amino acids **13**, **14** followed by deprotection, or direct condensation of PNC-amine **10** with amino acid chloride hydrochlorides **15**.

Compound **9** was condensed with *N*-carbobenzyloxy(CBZ)-L-alanine (**13a**) using iso butylchloroformate as an activating agent to afford **16a** in 50% yield after crystallization. The cleavage of the 2′-acetyl group in **16a** was effected with conc NH_4OH in MeOH, giving 2′-hydroxy compound **17a** in 44% yield. This cleavage reaction also produced the 2′-pyrrole carboxylate **18a** as a minor by-product. The formation of this 2′-ester is presumably due to transesterification under these conditions. Although migration of the carbamyl group of novobiocin from the 3′-position to the 2′-position is documented,[15] this is the first observation of the pyrrolecarbonyl group migration in the coumermycin series.[11] The benzyloxycarbonyl group in **17a** was removed by hydrogenolysis (Pd/C - EtOH) in the presence of dil HCl to produce (*S*)-alanine derivative **5a** in 85% yield. The addition of dil HCl was essential to avoid the formation of an unknown by-product during the hydrogenolysis. Using the method developed above, (*R*)-alanine derivative **5b** was prepared, in similar yield, *via* **16b** and **17b** from *N*-CBZ-D-alanine (**13b**). When this method was applied to *N*-CBZ-L-phenylglycine **14a** for the preparation of (*S*)-phenylglycine analog **6a**, the first condensation produced **19a** in 70% yield. However the second step, the cleavage reaction of the 2′-acetyl group in **19a** afforded a chromatographically inseparable mixture of 2′-hydroxy compound **20a** and 2′-pyrrole ester **21a** in ratio of about 5 to 1. Therefore, for the preparation of (2*S*)- and (2*R*)-aminophenylacetamide analogs **6a** and **6b**, an alternative and more direct approach has been developed. Condensation of **10** with L- and D-phenylglycyl chloride hydrochlorides[16] **15a** and **15b**, which were prepared from L- and D-phenyl-

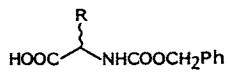


9 R=Ac
10 R=H

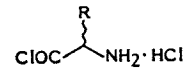


11 R=Ac
12 R=H

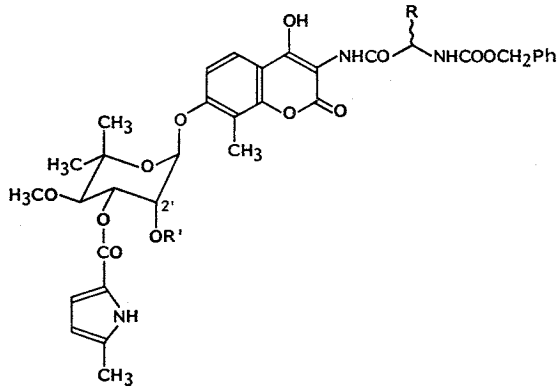
† PNC-Amine is 3-amino-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**10**). The coumermycin subunits are referred to as P, 5-methylpyrrole; N, noviose; and C, 4-hydroxy-8-methylcoumarin, see ref 11.



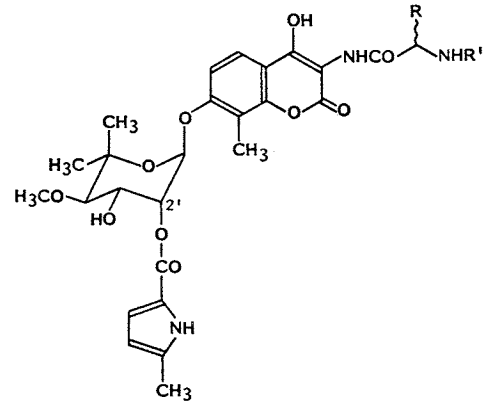
13a R=(*S*)-CH_3
13b R=(*R*)-CH_3
14a R=(*S*)-Ph



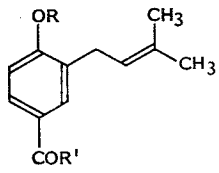
15a R=(*S*)-Ph
15b R=(*R*)-Ph



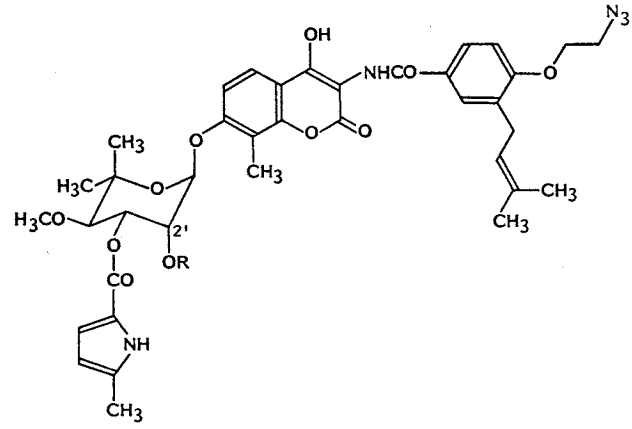
16a R=(*S*)-CH_3 R′=Ac
16b R=(*R*)-CH_3 R′=Ac
17a R=(*S*)-CH_3 R′=H
17b R=(*R*)-CH_3 R′=H
19a R=(*S*)-Ph R′=Ac
20a R=(*S*)-Ph R′=H



18a R=(*S*)-CH_3 R′=$COOCH_2Ph$
21a R=(*S*)-Ph R′=$COOCH_2Ph$



22 R=H R′=OEt
23 R=CH_2CH_2Br R′=OEt
24 R=$CH_2CH_2N_3$ R′=OEt
25 R=$CH_2CH_2N_3$ R′=OH
26 R=$CH_2CH_2N_3$ R′=Cl



27 R=Ac
28 R=H

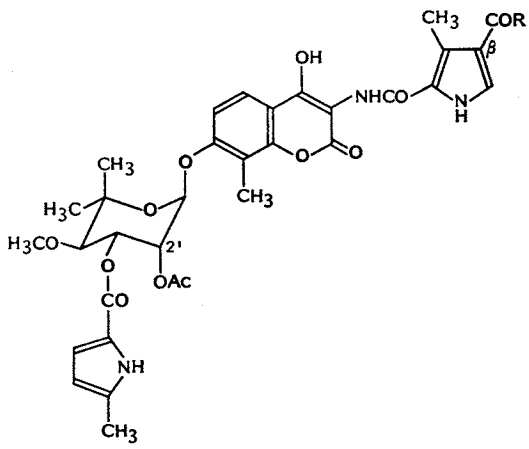
glycine hydrochlorides and PCl_5, furnished directly, in one step, **6a** and **6b** in 69 and 25% yield, respectively.

The aminoethoxybenzamido analog **7** was prepared using **9** as a starting intermediate. Treatment of ethyl 3-prenyl-4-hydroxybenzoate[17] (**22**) with 2-bromoethanol under the MITSUNOBU conditions[18] gave bromoethoxybenzoate **23** which was converted to the azide **24**. Hydrolysis of **24** to carboxylic acid **25** followed by treatment with oxalyl chloride provided 3-prenyl-4-(2-azidoethoxy)benzoyl chloride

(**26**) in *ca.* 50% overall yield from **22**. Condensation of **9** with **26** in pyridine afforded, in 33% yield, **27** which was converted with NH_4OH - MeOH to 2′-hydroxy compound **28** in 22% yield.

The azido and prenyl moieties of **28** were both hydrogenated with Pd-C in EtOH containing dil HCl, giving **7** in 66% yield as the hydrochloride.

Carboxypyrrole derivative **29a** was prepared in 16% yield as a minor by-product during a larger scale synthesis of 2′-acetyl oxazolocoumarin **11** which involved heating **1** with Ac_2O and pyridine.[14] Regiochemistry of the carboxy group on the pyrrole was established as β-carboxypyrrole, shown in **29a**, based on the work of KAWAGUCHI and co-workers.[2] This clearly indicates that the β-carboxyamide of the central pyrrole in **1** was more readily cleaved than the α-carboxyamide moiety under these conditions. This agrees with the long known fact that under acidic conditions β-carboxypyrrole esters are selectively cleaved in the presence of α-carboxy esters to pyrrole-β-carboxylic acids.[19] The 2′-acetyl group in **29a** was removed with NH_4OH - MeOH to furnish **8a** in 22% yield. The β-carbamoylpyrrole derivative **8b** was prepared in 10% yield from **29a**. Treatment of **29a** with *N*,*N*′-carbonyldiimidazole followed by liq NH_3 afforded **29b** which was converted to **8b** by the removal of the 2′-acetyl group with NH_4OH - MeOH. The low yield is partly due to the formation of a substantial amount of a polar by-product in the carbamoylation process.



29a R=OH
29b R=NH_2

In Vitro Antibacterial Activity

In vitro antibacterial activity of new semi-synthetic coumermycin analogs, **5a**, **5b**, **6a**, **6b**, **17a**, **17b**, **20a**, **7**, **8a** and **8b**, is summarized in the Table 1. Coumermycin A_1 (**1**), benzamido analog **3** and 4-hydroxy-3-(3-methylbutyl)benzamido analog **4** are included for comparison. The benzamido analog **3** was used traditionally as a standard for comparison and the 4-hydroxybenzamido derivative **4** was reported to be one of the most active compounds among the semi-synthetic coumermycin analogs.[11] Selected Gram-positive organisms listed in this table are *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, which includes methicillin-resistant strains, and a methicillin-resistant strain of *Staphylococcus epidermidis*. Methicillin-resistant strains of *S. aureus* A20700 and A25070 were chosen as representatives of strains which are sensitive to **1** and less sensitive to **1**, respectively.

The alanine and phenylglycine analogs, **5** and **6**, showed only modest antibacterial activity. The phenylglycine series **6** and **20a** were generally more active than the alanine series **5** and **17**. Interestingly, *N*-CBZ derivatives of (*S*)-alanine and (*S*)-phenylglycine analogs, **17a** and **20a**, showed much better anti-Staphylococcal activity than the parent amino acid analogs, **5a** and **6a**, and they possessed comparable antibacterial activity to the benzamido analog **3**. The aminoethoxybenzamido derivative **7** appeared to be much more soluble than **1** or the corresponding hydroxybenzamido analog **4**. Although **7** was generally less potent than **4**, **7** exhibited good antibacterial activity against Staphylococci

Table 1. *In vitro* antibacterial activity of new semi-synthetic coumermycin analogs, MIC (μg/ml).[a]

Compound	*S.pn.* A9585	*S.py.* A9604	*S.a.* A9537	*S.a.*/MR A20700	*S.a.*/MR A25070	*S.e.*/MR A25441
Coumermycin A_1 (**1**)	0.03	0.03	0.002	0.002	0.5	0.002
3	0.5	0.5	0.25	0.03	32	0.03
4	0.06	0.13	0.002	0.002	1	0.25
5a	4	4	8	4	32	2
5b	4	2	1	4	>32	2
17a	1	1	0.13	0.13	32	0.13
17b	4	2	8	8	>32	4
20a[b]	2	2	0.13	0.25	4	0.13
6a	2	2	2	1	32	0.25
6b	2	4	2	1	>32	4
7	2	2	0.13	0.5	0.5	0.13
8a	4	4	8	2	>32	2
8b	2	2	63	2	>125	1

[a] Determined by the 2-fold serial broth dilution method using nutrient broth, inoculum size: $1\sim5\times10^6$ cfu/ml. For methicillin-resistant strains, the incubation was carried out at 35°C for 24 hours (data from Microbiology Research Department, Wallingford, CT).

[b] Contaminated with *ca.* 17% of 2′-ester **21a**.

Abbreviations: *S.pn.*, *Streptococcus pneumoniae*; *S.py.*, *Streptococcus pyogenes*; *S.a.*, *Staphylococcus aureus*; *S.e.*, *Staphylococcus epidermidis*; MR, methicillin-resistant strain.

species including the methicillin-resistant strain A25070 which was resistant to most of the semi-synthetic analogs.

The carboxy- and carbamoyl-containing analogs, **8a** and **8b** were found to possess only modest antibacterial activity. Among new semi-synthetic analogs described here, **7** was found to be the most promising because of its improved solubility and uniformly good antibacterial activity, particularly against methicillin-resistant strains of Staphylococci species.

Experimental

MP's were determined on a MEL-TEMP apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. IR spectra were recorded on a Perkin-Elmer Model 1800 fourier transformation (FT)-IR spectrophotometer. The UV spectra were obtained with a Hewlett-Packard 8452A Diode Array spectrophotometer. The ^{1}H NMR spectra were taken with either a Bruker WM360 (360 MHz), a Bruker AM300 (300 MHz), or a Varian VXR-200 (200 MHz) NMR spectrometer. TMS or residual protonated solvent was used as an internal reference. Splitting patterns: s, singlet; d, doublet, t, triplet; dd, doublet of doublets; m, multiplet; br, broad. MS were obtained on either a Kratos MS25RFA (fast atom bombardment; FAB) using xenon atom bombardment or a Finnigan 4500 electron impact-chemical ionization (EI-CI) mass spectrometer. 3-Nitrobenzyl alcohol or thioglycerol was used as the supporting matrix in FAB-MS measurement. High resolution mass spectra (HR-MS) were recorded on a Kratos MS-50 instrument. THF was freshly distilled from sodium benzophenone ketyl. Freshly opened anhydrous diethyl ether (Aldrich) was used without further treatment. Pyridine was distilled from CaH_2 and stored over NaOH. Anhydrous solvents were obtained, drying over molecular sieves 4A (CH_2Cl_2) or 3A (DMF). *N*-CBZ-L-Phenylglycine[20] (**14a**) and ethyl 4-hydroxy-3-prenylbenzoate[17] (**22**) were prepared according to the reported procedures. The 2′-acetyl PNC-amine **9** and PNC-amine **10** were prepared as described in ref 14 and not purified. Analytical and preparative TLC was performed by using precoated plates (Silica gel 60A, MKGF, Whatman and Silica gel 60 F_{254}, E. Merck, respectively). The plates were visuallized by UV light. Column chromatography was run on open column of Silica gel

60 (70~230 mesh, E. Merck).

3-[2(*S*)-Benzyloxycarbonylaminopropionylamido]-4-hydroxy-8-methyl-7-[2-*O*-acetyl-3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**16a**)

To a stirred solution of *N*-CBZ-L-alanine (**13a**) (323 mg, 1.45 mmol) in anhydrous THF (10 ml) was injected *N*-methylmorpholine (160 μl, 1.45 mmol) and then isobutyl chloroformate (188 μl, 1.45 mmol) at $-15\sim-10$°C (ice-MeOH bath) under a nitrogen atmosphere. A few minutes after, to this mixture was added *N*-methylmorpholine (160 μl, 1.45 mmol) and then a solution of 2'-acetyl PNC-amine hydrochloride **9** (825 mg, 1.45 mmol) in anhydrous THF (10 ml) in 5 minutes. The mixture was stirred at ambient temperature for 3.5 hours. This was diluted with EtOAc, washed successively with dil $NaHCO_3$, dil HCl and then brine, dried over Na_2SO_4 and concentrated *in vacuo* to yield 1.124 g of a crude product. This was purified by column chromatography (SiO_2; EtOAc - CH_2Cl_2, 1:4 to 1:1), trituration with Et_2O and then recrystallization from EtOH - CH_2Cl_2 to give 537 mg (0.73 mmol, yield 50.3%) of **16a** as off-white crystals: MP 150~152°C; UV λ_{max}^{EtOH} nm (ε) 236 (17,200), 282 (28,700), 306 (16,600); IR (KBr) cm^{-1} 3300, 3000, 2950, 1710, 1640, 1610; ^1H NMR (360 MHz, $CDCl_3$) δ 1.22 (3H, s, 5'-CH_3), 1.39 (3H, s, 5'-CH_3), 1.50 (3H, d, J=7 Hz, 2'''-CH_3), 2.15 (3H, s, 2'-OAc), 2.31 (3H, s, 5''-CH_3), 2.33 (3H, s, 8-CH_3), 3.55 (3H, s, 4'-OCH_3), 3.57 (1H, d, J=10 Hz, 4'-H), 4.49 (1H, m, 2'''-H), 5.15 (2H, ABq, OCH_2), 5.2 (1H, br s, 2'''-NH), 5.53 (1H, t, J=2.5 Hz, 2'-H), 5.57 (1H, d, J=2.5 Hz, 1'-H), 5.75 (1H, dd, J=3 and 10 Hz, 3'-H), 5.95 (1H, t, J=3 Hz, 4''-H), 6.77 (1H, t, J=3 Hz, 3''-H), 7.14 (1H, d, J=9 Hz, 6-H), 7.35 (5H, s, Ar-Hs), 7.76 (1H, d, J=9 Hz, 5-H), 8.72 (1H, br s, 3-NH, exchanged with D_2O), 8.87 (1H, br s, 1''-H), 13.2 (1H, s, 4-OH, exchanged with D_2O); FAB-MS m/z 736 (M+H), 324, 108, 91.

Anal Calcd for $C_{37}H_{41}N_3O_{13}$: C 60.41, H 5.62, N 5.72.
Found: C 60.37, H 5.57, N 5.64.

3-[2(*S*)-Benzyloxycarbonylaminopropionylamido]-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**17a**)

To a suspension of **16a** (368 mg, 0.5 mmol) in MeOH (4 ml) was added conc NH_4OH - MeOH (1:9) (16 ml) to dissolve. This solution was stirred at room temperature for 3 days. Evaporation of the solvent to dryness gave white glassy solid which was purified by column chromatography (SiO_2; MeOH - CH_2Cl_2, 1:24) and precipitation from CH_2Cl_2 - hexane to give 154 mg (0.22 mmol, yield 44%) of **17a** as white powder: MP 130°C (dec); UV λ_{max}^{EtOH} nm (ε) 236 (17,100), 280 (27,200), 306 (16,400); IR (KBr) cm^{-1} 3400 (br), 3000, 2950, 1700, 1640, 1610; ^1H NMR (360 MHz, $CDCl_3$) δ 1.19 (3H, s, 5'-CH_3), 1.38 (3H, s, 5'-CH_3), 1.50 (3H, d, J=7.2 Hz, 2'''-CH_3), 2.29 (3H, s, 5''-CH_3), 2.31 (3H, s, 8-CH_3), 3.53 (3H, s, 4'-OCH_3), 3.63 (1H, d, J=10 Hz, 4'-H), 4.39 (1H, m, 2'-H), 4.50 (1H, m, 2'''-H), 5.14 (2H, ABq, OCH_2), 5.27 (1H, d, J=7 Hz, 2'''-NH), 5.61 (1H, d, J=2.3 Hz, 1'-H), 5.67 (1H, dd, J=3 and 10 Hz, 3'-H), 5.97 (1H, t, J=3 Hz, 4''-H), 6.86 (1H, t, J=3 Hz, 3''-H), 7.18 (1H, d, J=9 Hz, 6-H), 7.33 (5H, m, Ar-Hs), 7.76 (1H, d, J=9 Hz, 5-H), 8.72 (1H, br s, 3-NH, exchanged with D_2O), 9.01 (1H, br s, 1''-H), 13.2 (1H, s, 4-OH, exchanged with D_2O); FAB-MS m/z 694 (M+H), 412, 282, 108, 91.

Anal Calcd for $C_{35}H_{39}N_3O_{12}$: C 60.61, H 5.67, N 6.06.
Found: C 60.12, H 5.87, N 5.75.

During the column chromatography, **18a** was isolated (22 mg, 0.032 mmol, yield 6.3%) as white powder from the slightly faster moving fractions: MP 115~120°C (dec); UV λ_{max}^{EtOH} nm (ε) 234 (15,600), 282 (25,100), 320 (16,400); IR (KBr) cm^{-1} 3400 (br), 3000, 2950, 1700, 1640, 1610; ^1H NMR (360 MHz, $CDCl_3$) δ 1.16 (3H, s, 5'-CH_3), 1.38 (3H, s, 5'-CH_3), 1.49 (3H, d, J=7.2 Hz, 2'''-CH_3), 2.25 (3H, s, 5''-CH_3), 2.31 (3H, s, 8-CH_3), 3.39 (1H, d, J=9.5 Hz, 4'-H), 3.62 (3H, s, 4'-OCH_3), 4.45 (1H, m, with D_2O dd, J=4 and 10 Hz, 3'-H), 4.51 (1H, m, 2'''-H), 5.14 (2H, ABq, OCH_2), 5.25 (1H, d, J=6 Hz, 2'''-NH, exchanged with D_2O), 5.53 (1H, m, 2'-H), 5.66 (1H, d, J=1.5 Hz, 1'-H), 5.99 (1H, t, J=3 Hz, 4''-H), 6.92 (1H, t, J=3 Hz, 3''-H), 7.15 (1H, d, J=9 Hz, 6-H), 7.34 (5H, m, Ar-Hs), 7.74 (1H, d, J=9 Hz, 5-H), 8.73 (1H, br s, 3-NH, exchanged with D_2O), 8.97 (1H, br s, 1''-H, exchanged with D_2O); FAB-MS m/z 694 (M+H), 282, 108, 91.

3-[2(*S*)-Aminopropionylamido]-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**5a**)

A solution of **17a** (45 mg, 0.065 mmol) in abs EtOH (15 ml) was mixed with 1 N HCl (0.13 ml, 0.13 mmol) and 10% Pd-C (20 mg). This mixture was hydrogenated in a Parr apparatus at H_2, 2.5 kg/cm^2, room temperature for 1.5 hours, by which time TLC (MeOH - CH_2Cl_2, 1:9) indicated the reaction was complete. The catalyst was filtered over Celite and the filtrate was evaporated to yield yellowish powder which was purified by column chromatography (SiO_2; conc NH_4OH - MeOH - CH_2Cl_2, 3:27:70) to obtain 31 mg (0.055 mmol, yield 85%) of **5a** as white powder. This was dissolved in a minimum amount of 2-PrOH, removing any insoluble materials. The solvent was evaporated to give an analytical sample: MP 190~193°C (dec); UV λ_{max}^{EtOH} nm (ε) 238 (16,300), 280 (26,100), 304 (16,000); IR (KBr) cm^{-1} 3200 (br), 1685, 1640 (sh), 1605; ^{1}H NMR (360 MHz, CD_3OD) δ 1.19 (3H, s, 5'-CH_3), 1.36 (3H, s, 5'-CH_3), 1.62 (3H, d, J=7.1 Hz, 2'''-CH_3), 2.30 (3H, s, 5''-CH_3), 2.32 (3H, s, 8-CH_3), 3.52 (3H, s, 4'-OCH_3), 3.70 (1H, d, J=9.8 Hz, 4'-H), 4.03 (1H, q, J=7.1 Hz, 2'''-H), 4.27 (1H, t, J=2.6 Hz, 2'-H), 5.90 (1H, d, J=2.2 Hz, 1'-H), 5.67 (1H, dd, J=3 and 10 Hz, 3'-H), 5.94 (1H, d, J=3.6 Hz, 4''-H), 6.90 (1H, d, J=3.6 Hz, 3''-H), 7.14 (1H, d, J=9 Hz, 6-H), 7.82 (1H, d, J=9 Hz, 5-H); FAB-MS m/z 560 (M+H), 282, 108.

Anal Calcd for $C_{27}H_{33}N_3O_{10}\cdot 2\frac{1}{2}H_2O$: C 53.64, H 6.34, N 6.96.
Found: C 53.27, H 6.40, N 6.84.

Compounds **16b**, **17b**, **5b**, **19a** and **20a**

The title compounds were prepared from **9** using *N*-CBZ-D-alanine (**13b**) or *N*-CBZ-L-phenylglycine[20] (**14a**) as described above for the preparation of **16a**, **17a** and **5a**.

16b: Yield 63.8%; white foam; mp 116~119°C (dec); UV λ_{max}^{EtOH} nm (ε) 234 (16,400), 282 (27,600), 320 (18,500); IR (KBr) cm^{-1} 3300, 3000, 2950, 1710, 1640, 1610; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.22 (3H, s, 5'-CH_3), 1.39 (3H, s, 5'-CH_3), 1.50 (3H, d, J=7 Hz, 2'''-CH_3), 2.15 (3H, s, 2'-OAc), 2.31 (3H, s, 5''-CH_3), 2.33 (3H, s, 8-CH_3), 3.55 (3H, s, 4'-OCH_3), 3.58 (1H, d, J=10 Hz, 4'-H), 4.49 (1H, m, 2'''-H), 5.14 (2H, ABq, OCH_2), 5.22 (1H, br, 2'''-NH), 5.53 (1H, t, J=2.5 Hz, 2'-H), 5.57 (1H, d, J=2.5 Hz, 1'-H), 5.75 (1H, dd, J=3.6 and 9.8 Hz, 3'-H), 5.95 (1H, t, J=3 Hz, 4''-H), 6.77 (1H, t, J=3 Hz, 3''-H), 7.15 (1H, d, J=9 Hz, 6-H), 7.35 (5H, br s, Ar-Hs), 7.76 (1H, d, J=9 Hz, 5-H), 8.71 (1H, br s, 3-NH), 8.90 (1H, br s, 1''-H), 13.2 (1H, br s, 4-OH); FAB-MS m/z 736 (M+H), 324, 108, 91.

Anal Calcd for $C_{37}H_{41}N_3O_{13}$: C 60.41, H 5.62, N 5.72.
Found: C 60.55, H 5.86, N 5.49.

17b: Yield 39.4%; white solid; mp 130~135°C (dec); UV λ_{max}^{EtOH} nm (ε) 236 (16,500), 280 (26,600), 314 (17,200); IR (KBr) cm^{-1} 3400 (br), 3000, 2950, 1700, 1640, 1610; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.19 (3H, s, 5'-CH_3), 1.39 (3H, s, 5'-CH_3), 1.50 (3H, d, J=7 Hz, 2'''-CH_3), 2.32 (6H, s, 5''-CH_3, 8-CH_3), 3.53 (3H, s, 4'-OCH_3), 3.63 (1H, d, J=9.1 Hz, 4'-H), 4.39 (1H, m, 2'-H), 4.49 (1H, m, 2'''-H), 5.15 (2H, ABq, OCH_2), 5.22 (1H, d, J=6 Hz, 2'''-NH), 5.62 (1H, d, J=2.6 Hz, 1'-H), 5.67 (1H, dd, J=2.8 and 9.8 Hz, 3'-H), 5.98 (1H, t, J=3 Hz, 4''-H), 6.87 (1H, t, J=3 Hz, 3''-H), 7.19 (1H, d, J=9 Hz, 6-H), 7.35 (5H, br s, Ar-Hs), 7.76 (1H, d, J=9 Hz, 5-H), 8.71 (1H, br s, 3-NH), 8.96 (1H, br s, 1''-H), 13.2 (1H, br s, 4-OH); FAB-MS m/z 694 (M+H), 412, 282, 108, 91.

Anal Calcd for $C_{35}H_{39}N_3O_{12}\cdot\frac{1}{2}H_2O$: C 59.83, H 5.74, N 5.99.
Found: C 59.62, H 5.81, N 5.91.

5b: Yield 85%; off-white solid; mp 191~193°C (dec); UV λ_{max}^{EtOH} nm (ε) 238 (17,400), 280 (27,800); IR (KBr) cm^{-1} 3400 (br), 1685, 1650 (sh), 1610; ^{1}H NMR (360 MHz, CD_3OD) δ 1.19 (3H, s, 5'-CH_3), 1.36 (3H, s, 5'-CH_3), 1.63 (1H, d, J=7 Hz, 2'''-CH_3), 2.30 (3H, s, 5''-CH_3), 2.32 (3H, s, 8-CH_3), 3.52 (3H, s, 4'-OCH_3), 3.71 (1H, d, J=9.9 Hz, 4'-H), 4.05 (1H, q, J=7 Hz, 2'''-H), 4.27 (1H, t, J=2.6 Hz, 2'-H), 5.59 (1H, d, J=2.5 Hz, 1'-H), 5.67 (1H, dd, J=3 and 10 Hz, 3'-H), 5.94 (1H, d, J=3.5 Hz, 4''-H), 6.90 (1H, d, J=3.5 Hz, 3''-H), 7.15 (1H, d, J=9 Hz, 6-H), 7.82 (1H, d, J=9 Hz, 5-H); FAB-MS m/z 560 (M+H), 282, 279, 108.

19a: Yield 70%; white solid; mp 130~136°C (dec); UV λ_{max}^{EtOH} nm (ε) 282 (27,400), 316 (17,700); IR (KBr) cm^{-1} 3340, 3000, 2950, 1710, 1640, 1610; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (3H, s, 5'-CH_3), 1.96 (3H, s, 5'-CH_3), 2.14 (3H, s, 2'-OAc), 2.30 (6H, s, 5''-CH_3, 8-CH_3), 3.54 (3H, s, 4'-OCH_3),

3.55 (1H, d, J=10 Hz, 4′-H), 5.13 (2H, s, OCH_2), 5.46 (1H, br, 2‴-H), 5.53 (1H, m, 2′-H), 5.56 (1H, d, J=2 Hz, 1′-H), 5.63 (1H, br, 2‴-NH; not exchanged, but shifted to 5.66 with D_2O), 5.64 (1H, dd, J=3 and 10 Hz, 3′-H), 5.94 (1H, t, J=3 Hz, 4″-H), 6.76 (1H, t, J=3 Hz, 3″-H), 7.13 (1H, d, J=9 Hz, 6-H), 7.3~7.4 (10H, m, Ar-Hs), 7.74 (1H, d, J=9 Hz, 5-H), 8.65 (1H, br, 3-NH, exchanged with D_2O), 8.94 (1H, br, 1″-H), 12.95 (1H, s, 4-H, exchanged with D_2O); FAB-MS m/z 798 (M+H), 324, 108, 91.

Anal Calcd for $C_{42}H_{43}N_3O_{13}\cdot\frac{1}{2}H_2O$: C 62.53, H 5.50, N 5.21.
Found: C 62.45, H 5.62, N 5.34.

20a: This material was contaminated with *ca.* 17% of 2′-pyrrolecarboxylate **21a**: Yield 55%; white solid; mp 167~180°C (dec); UV λ_{max}^{EtOH} nm (ε) 280 (28,200), 306 (15,600); IR (KBr) cm^{-1} 3300, 3000, 2950, 1700, 1640, 1610; ^1H NMR (360 MHz, CD_3OD) δ 1.16 (3H, s, 5′-CH_3), 1.35 (3H, s, 5′-CH_3), 2.28, 2.29 (2×3H, 2s, 5″-CH_3, 8-CH_3), 3.52 (3H, s, 4′-OCH_3), 3.63 (s, 4′-OCH_3 of **21a**), 3.70 (1H, d, J=10 Hz, 4′-H), 4.28 (1H, t, J=2.5 Hz, 2′-H), 4.40 (dd, J=3 and 9 Hz, 3′-H of **21a**), 5.10 (2H, s, OCH_2), 5.36 (t, J=3 Hz, 2′-H of **21a**), 5.55 (1H, s, 2‴-H), 5.60 (1H, d, J=2 Hz, 1′-H), 5.66 (1H, dd, J=3 and 10 Hz, 3′-H), 5.94 (1H, d, J=3.5 Hz, 4″-H), 6.91 (1H, d, J=3.5 Hz, 3″-H), 7.13 (1H, d, J=9 Hz, 6-H), 7.3~7.6 (10H, m, Ar-Hs), 7.80 (1H, d, J=9 Hz, 5-H); FAB-MS m/z 756 (M+H), 282, 108, 91; HR-MS calcd for $C_{40}H_{42}N_3O_{12}$ $(M+H)^+$ 756.2786, found 756.2759.

Anal Calcd for $C_{40}H_{41}N_3O_{12}\cdot 2H_2O$: C 60.68, H 5.73, N 5.31.
Found: C 60.81, H 5.33, N 5.90.

(*R*)-Phenylglycyl Chloride Hydrochloride (**15b**)

The title compound was prepared by a modification of the method reported in ref 16. To a stirred slurry of (*R*)-phenylglycine (15.1 g, 0.1 mol) in CH_2Cl_2 (300 ml) was bubbled dry HCl gas vigorously while cooling in an ice-bath for 20 minutes. To this mixture was added then PCl_5 (25 g, 0.12 mol) under a dry argon atmosphere and stirred for 30 minutes at 0~5°C. The cooling bath was removed and the mixture stirred for 5 hours. The product was filtered, washed with dry CH_2Cl_2 under a dry argon atmosphere and dried in a desiccator containing P_2O_5 under a reduced pressure overnight to obtain 20.1 g (0.0975 mol, yield 97.5%) of **15b** as white crystals: IR (Nujol) cm^{-1} 1770 (COCl).

Anal Calcd for $C_8H_8ClNO\cdot HCl$: C 46.63, H 4.41, N 6.80, Cl 34.41.
Found: C 46.86, H 4.49, N 6.89, Cl 34.24.

The title compound was hygroscopic and used immediately in the subsequent reaction.

The (*S*)-isomer **15a** was similarly prepared in 94% yield from (*S*)-phenylglycine: IR (KBr) cm^{-1} 1772 (COCl).

3-[2(*S*)-Amino-2-phenylacetamido]-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)-noviosyloxy]coumarin Hydrochloride (**6a**)

To a stirred suspension of PNC-amine hydrochloride **10** (468 mg, 0.892 mmol) in CH_2Cl_2 (40 ml) was added *N*,*N*-dimethylaniline (260 μl, 2.03 mmol; dried over KOH) and then **15a** (187 mg, 0.9 mmol) in portions at 0~5°C under a dry argon atmosphere. The mixture was stirred for 15 minutes and then anhydrous THF (13 ml) was added. The mixture was stirred at 0~5°C for 4.5 hours and an additional hour after removing the cooling bath. The solvents were evaporated and the crude residue was purified by column chromatography (SiO_2; concd NH_4OH - MeOH - CH_2Cl_2, 1:9:90 to 2:18:80) to yield 384 mg (0.618 mmol, yield 69.4%) of **6a** as a free base form. A portion of this material (207 mg, 0.333 mmol) was dissolved in abs EtOH, acidified with conc HCl (28 μl), the EtOH evaporated and the residue triturated with anhydrous Et_2O to obtain 134 mg (0.204 mmol) of **6a** as white powder: MP 180~210°C (dec); UV λ_{max}^{EtOH} nm (ε) 280 (26,300), 308 (16,000); IR (KBr) cm^{-1} 1690, 1610; ^1H NMR (360 MHz, CD_3OD) δ 1.16 (3H, s, 5′-CH_3), 1.36 (3H, s, 5′-CH_3), 2.29 (3H, s, 5″-CH_3), 2.32 (3H, s, 8-CH_3), 3.52 (3H, s, 4′-OCH_3), 3.71 (1H, d, J=10 Hz, 4′-H), 4.28 (1H, t, J=3 Hz, 2′-H), 5.29 (1H, s, 1‴-H), 5.63 (1H, d, J=2.5 Hz, 1′-H), 5.66 (1H, dd, J=3 and 10 Hz, 3′-H), 5.94 (1H, d, J=3 Hz, 4″-H), 6.91 (1H, d, J=3 Hz, 3″-H), 7.26 (1H, d, J=9 Hz, 6-H), 7.53 (3H, m, Ar-Hs), 7.66 (3H, m, Ar-Hs), 7.81 (1H, d, J=9 Hz, 5-H); FAB-MS m/z 622 (M+H), 341, 282.

Anal Calcd for $C_{32}H_{35}N_3O_{10}\cdot HCl\cdot 2H_2O$: C 55.38, H 5.81, N 6.06.
Found: C 55.18, H 5.56, N 6.00.

3-[2(*R*)-Amino-2-phenylacetamido]-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)-noviosyloxy]coumarin Hydrochloride (**6b**)

The title compound was prepared from **15b** by the method described for the *S*-isomer **6a**: MP 150~180°C (dec); UV λ_{max}^{EtOH} nm (ε) 280 (26,300), 310 (16,300); IR (KBr) cm^{-1} 1700, 1610; ^1H NMR (360 MHz, CD_3OD) δ 1.16 (3H, s, 5'-CH_3), 1.36 (3H, s, 5'-CH_3), 2.30 (3H, s, 5''-CH_3), 2.32 (3H, s, 8-CH_3), 3.52 (3H, s, 4'-OCH_3), 3.71 (1H, d, *J*=10 Hz, 4'-H), 4.28 (1H, t, *J*=3 Hz, 2'-H), 5.31 (1H, s, 2'''-H), 5.64 (1H, d, *J*=3 Hz, 1'-H), 5.65 (1H, dd, *J*=3 and 10 Hz, 3'-H), 5.94 (1H, d, *J*=3 Hz, 4''-H), 6.91 (1H, d, *J*=3 Hz, 3''-H), 7.26 (1H, d, *J*=9 Hz, 6-H), 7.53 (3H, m, Ar-Hs), 7.67 (2H, m, Ar-Hs), 7.80 (1H, d, *J*=9 Hz, 5-H); FAB-MS *m/z* 622 (M+H), 341, 282, 108.

Ethyl 4-(2-Bromoethoxy-3-(3-methyl-2-butenyl)benzoate (**23**)

To a stirred solution of ethyl 4-hydroxy-3-(3-methyl-2-butenyl)benzoate[17] (**22**) (2.34 g, 10.0 mmol) and triphenylphosphine (2.89 g, 11.0 mmol) in anhydrous THF (50 ml) was added in 5 minutes a solution of diethyl azodicarboxylate[18] (DEAD, 1.82 ml, 11.0 mmol) in anhydrous THF (10 ml). To this mixture was injected 2-bromoethanol (0.75 ml, 10 mmol) in 15 minutes under N_2 at 20°C. The mixture was stirred at room temperature for 20 hours. The solvent was evaporated to dryness and extracted with anhydrous Et_2O (*ca.* 30 ml), removing the insoluble crystals. The filtrate was concentrated *in vacuo* to a crude oil. This was purified by column chromatography (SiO_2; CH_2Cl_2) to obtain 2.38 g (6.98 mmol, yield 69.8%) of **23** as a clear oil: Rf 0.55 (EtOAc - hexane, 1 : 4); IR (film) cm^{-1} 1710 (ester), 1610; ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (3H, t, *J*=7 Hz, CH_3), 1.74 (6H, s, 3''-$(CH_3)_2$), 3.37 (2H, d, *J*=7 Hz, 1''-H_2), 3.68 (2H, t, *J*=6 Hz, 2'-H_2), 4.34 (2H, q, *J*=7 Hz, $COOCH_2$), 4.36 (2H, t, *J*=6 Hz, 1'-H_2), 5.31 (1H, t, *J*=7 Hz, 2''-H), 6.80 (1H, d, *J*=8 Hz, 5-H), 7.85 (1H, s, 2-H), 7.87 (1H, d, *J*=8 Hz, 6-H).

Ethyl 4-(2-Azidoethoxy)-3-(3-methyl-2-butenyl)benzoate (**24**)

A mixture of **23** (1.71 g, 5.0 mmol) and sodium azide (603 mg, 9.0 mmol) in DMF (15 ml) was heated at 60~70°C for 5 hours under N_2. This was diluted with EtOAc, washed with H_2O (×2) and brine. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2; EtOAc - hexane, 1 : 4) to give 1.29 g (4.26 mmol, yield 85.2%) of **24** as a colorless oil; Rf 0.45 (EtOAc - hexane, 1 : 4); IR (film) cm^{-1} 2110 (N_3), 1710 (ester), 1610; ^1H NMR (200 MHz, $CDCl_3$) δ 1.39 (3H, t, *J*=7 Hz, CH_3), 1.74 (6H, d, *J*=7 Hz, 3''-$(CH_3)_2$), 3.38 (2H, d, *J*=7 Hz, 1''-H_2), 3.66 (2H, t, *J*=5 Hz, 2'-H_2), 4.22 (2H, t, *J*=5 Hz, 1'-H_2), 4.32 (2H, q, *J*=7 Hz, $COOCH_2$), 5.33 (1H, t, *J*=7 Hz, 2''-H), 6.83 (1H, d, *J*=8 Hz, 5-H), 7.87 (1H, s, 2-H), 7.89 (1H, d, *J*=8 Hz, 6-H).

4-(2-Azidoethoxy)-3-(3-methyl-2-butenyl)benzoic Acid (**25**)

A suspension of **24** (1.21 g, 4.00 mmol) in MeOH (45 ml) was mixed with 1 N NaOH (15 ml) and the mixture was heated at reflux for 7.5 hours. The MeOH was evaporated *in vacuo* and the residue was dissolved in H_2O, removing any insoluble materials. This clear filtrate was acidified with 6 N HCl (*ca.* 3 ml) in an ice-bath. The precipitate was filtered and dried *in vacuo* to give 950 mg (3.45 mmol, yield 86.4%) of **25** as off-white powder: IR (film) cm^{-1} 2110 (N_3), 1670 (COOH), 1605; ^1H NMR (200 MHz, $CDCl_3$) δ 1.73 (3H, s, 3''-CH_3), 1.78 (3H, s, 3''-CH_3), 3.39 (2H, d, *J*=7 Hz, 1''-H_2), 3.67 (2H, t, *J*=5 Hz, 2'-H_2), 4.24 (2H, t, *J*=5 Hz, 1'-H_2), 5.34 (1H, t, *J*=7 Hz, 2''-H), 6.87 (1H, d, *J*=8.6 Hz, 5-H), 7.9~8.0 (2H, m, 2-H, 6-H); MS *m/z* 276 (M+H), 248, 192.

Anal Calcd for $C_{14}H_{17}N_3O_3$: C 61.08, H 6.23, N 15.27.
Found: C 61.12, H 6.25, N 15.20.

4-(2-Azidoethoxy)-3-(3-methyl-2-butenyl)benzoyl Chloride (**26**)

To a stirred solution of **25** (647 mg, 2.35 mmol) in CH_2Cl_2 (27 ml) was injected oxalyl chloride (215 μl, 2.44 mmol) at room temperature, and the mixture was heated at reflux for 2 hours. The

solvent was evaporated *in vacuo* to dryness to yield yellowish liquid: IR (film) cm^{-1} 2110 (N_3), 1740 (COCl), 1600; ^1H NMR (200 MHz, $CDCl_3$) δ 1.71 (3H, s, 3″-CH_3), 1.77 (3H, s, 3″-CH_3), 3.38 (2H, d, J=7 Hz, 1″-H_2), 3.68 (2H, t, J=5 Hz, 2′-H_2), 4.26 (2H, t, J=5 Hz, 1′-H_2), 5.30 (1H, t, J=7 Hz, 2″-H), 6.88 (1H, d, J=9 Hz, 5-H), 7.90 (1H, d, J=2 Hz, 2-H), 8.00 (1H, dd, J=2 and 9 Hz, 6-H).

3-[4-(2-Azidoethoxy)-3-(3-methyl-2-butenyl)]benzamido-4-hydroxy-8-methyl-7-[2-*O*-acetyl-3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**27**)

To a stirred solution of **9** (820 mg, 1.45 mmol) in pyridine (20 ml) was added the acid chloride **26** (470 mg, 1.60 mmol) dropwise through a syringe at −30°C under an atmosphere of argon. The cooling bath was removed and the mixture was stirred at ambient temperature for 20 hours. This was diluted with CH_2Cl_2, washed successively with cold 3 N HCl, satd $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2; MeOH - CH_2Cl_2, 1 : 99 to 3 : 97) to yield 385 mg (0.489 mmol, yield 33.7%) of **27** as yellowish solid: MP 115~120°C; IR (film) cm^{-1} 3310, 3000, 2950, 2210 (N_3), 1755 (OAc), 1690, 1640, 1610; ^1H NMR (360 MHz, $CDCl_3$) δ 1.23 (3H, s, 5′-CH_3), 1.40 (3H, s, 5′-CH_3), 1.72 (3H, s, prenyl CH_3), 1.77 (3H, s, prenyl CH_3), 2.15 (3H, s, OAc), 2.31 (3H, s, 5″-CH_3), 2.36 (3H, s, 8-CH_3), 3.39 (1H, d, J=7 Hz, $ArCH_2$), 3.55 (3H, s, 4′-OCH_3), 3.57 (1H, d, partially seen, 4′-H), 3.66 (2H, t, J=5 Hz, CH_2N_3), 4.23 (2H, t, J=5 Hz, OCH_2), 5.32 (1H, t, J=7 Hz, CH=), 5.54 (1H, m, 2′-H), 5.59 (1H, d, J=2 Hz, 1′-H), 5.76 (1H, dd, J=3.6 and 10 Hz, 3′-H), 5.96 (1H, t, J=3 Hz, 4″-H), 6.77 (1H, t, J=3 Hz, 3″-H), 6.89 (1H, d, J=9 Hz, 5‴-H), 7.17 (1H, d, J=9 Hz, 6-H), 7.74 (1H, d, J=2 Hz, 2‴-H), 7.79 (1H, dd, J=2 and 9 Hz, 6‴-H), 7.82 (1H, d, J=9 Hz, 5-H), 8.74 (1H, s, 3-NH), 8.86 (1H, br s, 1″-H), 14.0 (1H, s, 4-OH); FAB-MS m/z 787 (M), 324, 258, 108; HR-MS calcd for $C_{40}H_{46}N_5O_{12}$ $(M+H)^+$ 788.3143, found 788.3122.

3-[4-(2-Azidoethoxy)-3-(3-methyl-2-butenyl)]benzamido-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**28**)

To a stirred suspension of **27** (123 mg, 0.156 mmol) in MeOH (20 ml) was added conc NH_4OH (6 ml) and the mixture was stirred at room temperature for 20 hours. The solvent was evaporated *in vacuo* to dryness and the residue was purified by preparative TLC (SiO_2; EtOAc - CH_2Cl_2, 1 : 4) to give 25 mg (0.034 mmol, yield 22%) of **28** as white solid: IR (KBr) cm^{-1} 3400 (OH), 2110 (N_3), 1690, 1640, 1610; ^1H NMR (360 MHz, $CDCl_3$) δ 1.20 (3H, s, 5′-CH_3), 1.39 (3H, s, 5′-CH_3), 1.72 (3H, s, prenyl CH_3), 1.77 (3H, s, prenyl CH_3), 2.32 (3H, s, 5″-CH_3), 2.34 (3H, s, 8-CH_3), 3.39 (2H, d, J=7 Hz, Ar-CH_2), 3.53 (3H, s, 4′-OCH_3), 3.63 (1H, d, J=9.5 Hz, 4′-H), 3.66 (2H, t, J=5 Hz, CH_2N_3), 4.23 (2H, t, J=5 Hz, OCH_2), 4.40 (1H, m, 2′-H), 5.32 (1H, t, J=7 Hz, CH=), 5.63 (1H, d, J=2.5 Hz, 1′-H), 5.69 (1H, dd, J=3 and 9.5 Hz, 3′-H), 5.98 (1H, t, J=3 Hz, 4″-H), 6.88 (1H, t, overlapped with 5‴-H, 3″-H), 6.89 (1H, d, J=9 Hz, 5‴-H), 7.22 (1H, d, J=9 Hz, 6-H), 7.74 (1H, d, J=2 Hz, 2‴-H), 7.77 (1H, dd, J=2 and 9 Hz, 6‴-H), 7.83 (1H, d, J=9 Hz, 5-H), 8.74 (1H, s, 3-NH), 8.91 (1H, br s, 1″-H), 14.0 (1H, s, 4-OH); FAB-MS m/z 745 (M), 282, 258, 108.

3-[4-(2-Aminoethoxy)-3-(3-methylbutyl)]benzamido-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin Hydrochloride (**7**)

A suspension of **28** (57 mg, 0.076 mmol) in abs EtOH (40 ml) was mixed with 1 N HCl (127 ml) and 10% Pd-C (57 mg). This mixture was hydrogenated in a Parr apparatus at 2.8 kg/cm^2 of H_2 and at room temperature for 2.5 hours. The catalyst was filtered (Celite) and the filtrate concentrated to dryness. The residue was purified by preparative TLC (SiO_2; conc NH_4OH - MeOH - CH_2Cl_2, 1 : 9 : 40) to give white solid which was dissolved in 6 N HCl (13 μl) and abs EtOH (10 ml). Evaporation of the solvents followed by trituration with anhydrous Et_2O gave 38 mg (0.050 mmol, yield 66%) of **7** as white powder: MP 140~150°C (dec); UV λ_{max}^{EtOH} nm (ε) 250 (17,300), 278 (22,500), 308 (13,500); IR (KBr) cm^{-1} 3400, 1700, 1640, 1610; ^1H NMR (360 MHz, DMSO-d_6) δ 0.95 (6H, d, J=6.6 Hz, $(CH_3)_2$), 1.08 (3H, s, 5′-CH_3), 1.29 (3H, s, 5′-CH_3), 1.47 (2H, q, J=8 Hz, CH_2), 1.60 (1H, m, CH), 2.24, 2.25 (6H, 2s, 5″-CH_3, 8-CH_3), 2.69 (2H, t, J=8 Hz, Ar-CH_2), 3.46 (3H, s, 4′-OCH_3), 3.65 (1H, d, J=10 Hz, 4′-H), 4.16 (1H, m, 2′-H), 4.25 (2H, t, J=5 Hz, OCH_2), 5.48 (1H, dd, J=3 and 10 Hz, 3′-H), 5.61 (1H, d, J=2 Hz, 1′-H), 5.69 (1H, d, J=5 Hz, 2′-OH, exchanged with D_2O), 5.92 (1H, br s,

4″-H), 6.77 (1H, t, J=2.5 Hz, 3″-H), 7.07 (1H, d, J=8.5 Hz, 5‴-H), 7.19 (1H, d, J=9 Hz, 6-H), 7.75 (1H, d, J=9 Hz, 5-H), 7.85 (1H, s, 2‴-H), 7.87 (1H, d, J=9 Hz, 6‴-H), 8.15 (br, D_2O exchangeable), 9.34 (1H, s, 1″-H); FAB-MS m/z 722 (M+H−HCl), 440, 282, 234, 108; HR-MS calcd for $C_{38}H_{48}N_3O_{11}$ (M+H−HCl) 722.3289, found 722.3306.

3-(4-Carboxy-3-methyl-2-pyrrolylcarbonylamino)-4-hydroxy-8-methyl-7-[2-*O*-acetyl-3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**29a**)

To a solution of **1** (55.5 g, 50 mmol) in pyridine (300 ml) was added acetic anhydride (100 ml) and the mixture heated at reflux for 3 hours. After cooling, this mixture was poured slowly onto a stirred mixture of 3 N HCl (2 liters) and ice cubes (2 liters). The brown precipitate formed was filtered, washed with H_2O and dried *in vacuo*. This solid was dissolved in warm CH_2Cl_2 (500 ml) and placed on a pad consisting of a thin-layer of Celite (*ca.* 50 g) over silica gel (500 g). After eluting with EtOAc - CH_2Cl_2 (1 : 4) to obtain oxazole **11** (26 g), the pad was eluted with MeOH - EtOAc (1 : 1, 2 liters). All MeOH - EtOH eluents were combined, and concentrated *in vacuo* to a volume of approximately 500 ml. Insoluble materials were removed by filtration through a Celite pad and the filtrate was concentrated to a volume of *ca.* 300 ml. This was left to stand at room temperature for 48 hours. The crystalline precipitate was collected, washed with EtOAc (3×100 ml) and dried to give 5.50 g (8.1 mmol, yield 16%) of the title compound **29a** as off-white powder. An analytical sample was obtained by repeating the precipitation process described above: MP 260°C (dec); Rf 0.65 (CH_2Cl_2 - MeOH - AcOH, 94 : 4 : 2); UV λ_{max}^{EtOH} nm (ε) 278 (30,600), 334 (24,300); IR (KBr) cm^{-1} 3300 (br), 3000, 2930, 1700, 1630, 1600; ^1H NMR (360 MHz, DMSO-d_6) δ 1.15 (3H, s, 5′-CH_3), 1.37 (3H, s, 5′-CH_3), 2.11 (3H, s, 2′-OAc), 2.23, 2.25 (6H, 2s, 5″-CH_3, 8-CH_3), 2.56 (3H, s, 3‴-CH_3), 3.49 (3H, s, 4-OCH_3), 3.54 (1H, d, J=9 Hz, 4′-H), 5.41 (1H, t, J=3 Hz, 2′-H), 5.58 (1H, dd, J=3 and 9 Hz, 3′-H), 5.81 (1H, d, J=3 Hz, 1′-H), 5.91 (1H, br s, 4″-H), 6.73 (1H, t, J=2.5 Hz, 3″-H), 7.19 (1H, d, J=9 Hz, 6-H), 7.52 (1H, d, J=3 Hz, 5‴-H), 7.76 (1H, d, J=9 Hz, 5-H), 8.71 (1H, s, 3-NH, exchanged with D_2O), 11.68 (1H, s, 1″-H), 11.88 (1H, d, J=3 Hz, 1‴-H, exchanged with D_2O), 11.93 (1H, br s, 4-OH, exchanged with D_2O), 12.26 (1H, br, 4‴-COOH, exchanged with D_2O); FAB-MS m/z 682 (M+H), 324.

Anal Calcd for $C_{33}H_{35}N_3O_{13}\cdot H_2O$: C 56.66, H 5.34, N 6.01.
Found: C 56.51, H 5.16, N 5.71.

3-(4-Carboxy-3-methyl-2-pyrrolylcarbonylamino)-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**8a**)

A solution of 2′-acetate **29a** (1.50 g, 2.20 mmol) in MeOH (20 ml) and conc NH_4OH (3 ml) was stirred at room temperature for 24 hours by which time TLC indicated the reaction was complete. This was concentrated to a volume of *ca.* 10 ml and then poured onto a cold stirred 2 N HCl solution (200 ml). The resulting precipitate was filtered, washed with H_2O and dried to give 1.20 g of solid. This solid was dissolved in MeOH (50 ml) and EtOAc (50 ml) with heating and treated with activated carbon (1 g). The carbon was filtered while it was hot, washed with MeOH - EtOAc (1 : 1) and the filtrate and washings concentrated to a volume of *ca.* 35 ml. The insolubles were filtered and the filtrate concentrated to a volume of *ca.* 20 ml. The resulting precipitate was filtered, washed with EtOAc and dried to give 315 mg (0.492 mmol, yield 22.4%) of **8a** as off-white powder: MP 200~240°C (dec); Rf 0.45 (CH_2Cl_2 - MeOH - AcOH, 94 : 4 : 2); UV λ_{max}^{EtOH} nm (ε) 276 (27,200), 336 (23,700); IR (KBr) cm^{-1} 3390, 3220, 3000, 2950, 1700, 1640, 1600; ^1H NMR (360 MHz, DMSO-d_6) δ 1.08 (3H, s, 5′-CH_3), 1.30 (3H, s, 5′-CH_3), 2.24, 2.26 (6H, 2s, 5″-CH_3, 8-CH_3), 2.56 (3H, s, 3‴-CH_3), 3.47 (3H, s, 4′-OCH_3), 3.65 (1H, d, J=10 Hz, 4′-H), 4.17 (1H, br s, 2′-H), 5.48 (1H, dd, J=3 and 10 Hz, 3′-H), 5.62 (1H, d, J=2 Hz, 1′-H), 5.68 (1H, br, 2′-OH), 5.92 (1H, s, 4″-H), 6.77 (1H, t, J=3 Hz, 3″-H), 7.21 (1H, d, J=9 Hz, 6-H), 7.53 (1H, d, J=3 Hz, 5‴-H), 7.75 (1H, d, J=9 Hz, 5-H), 8.73 (1H, s, 3-NH, exchanged with D_2O), 11.64 (1H, s, 1″-H), 11.90 (1H, d, J=3 Hz, 1‴-H, exchanged with D_2O), 11.95, 12.25 (2H, 2br, 4-OH, 4‴-COOH, exchanged with D_2O); FAB-MS m/z 640 (M+H), 282, 108.

Anal Calcd for $C_{31}H_{33}N_3O_{12}\cdot H_2O$: C 56.62, H 5.37, N 6.39.
Found: C 56.48, H 5.38, N 5.99.

3-(4-Carbamyl-3-methyl-2-pyrrolylcarbonylamino)-4-hydroxy-8-methyl-7-[2-*O*-acetyl-3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**29b**)

To a stirred solution of **29a** (2.00 g, 2.93 mmol) in dry DMF (20 ml, dried over molecular sieves 3A) was added *N,N'*-carbonyldiimidazole (1.20 g, 7.4 mmol) and the mixture stirred at room temperature for 52 hours. Dry ammonia gas was bubbled in with cooling (ice bath) for 2 hours. The ice bath was removed and the mixture stirred for an additional hour. The solvent was evaporated *in vacuo* and the residue was taken up in wet EtOAc (200 ml) to which AcOH (3 ml) was added. The organic layer was washed with H_2O ($\times 3$) then with brine, dried (Na_2SO_4) and concentrated to dryness. The residue was triturated with Et_2O to give 1.40 g of solid. This was purified by column chromatography (SiO_2; MeOH - CH_2Cl_2, 1:19 to 1:4) followed by precipitation from MeOH - CH_2Cl_2 to obtain 280 mg (0.411 mmol, yield 14%) of **29b** as powder: MP 222~224°C (dec); Rf 0.21 (CH_2Cl_2 - MeOH - HCOOH, 94:4:2); UV $\lambda_{max}^{50\%\ EtOH}$ nm (ε) 282 (34,700); IR (KBr) cm^{-1} 3390, 3000, 2950, 1700, 1640, 1600; ^1H NMR (360 MHz, DMSO-d_6) δ 1.08 (3H, s, 5'-CH_3), 1.15 (3H, s, 5'-CH_3), 2.11 (3H, s, 2'-OAc), 2.23 (3H, s, 5''-CH_3 or 8-CH_3), 2.25 (3H, s, 5''-CH_3 or 8-CH_3), 2.57 (3H, s, 3'''-CH_3), 3.49 (3H, s, 4'-OCH_3), 3.54 (1H, d, J=9 Hz, 4'-H), 5.41 (1H, t, J=3 Hz, 2'-H), 5.58 (1H, dd, J=3 and 9 Hz, 3'-H), 5.81 (1H, d, J=2.7 Hz, 1'-H), 5.91 (1H, br s, 4''-H), 6.73 (1H, t, J=2.3 Hz, 3''-H), 6.78 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 7.20 (1H, d, J=9 Hz, 6-H), 7.29 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 7.54 (1H, d, J=3 Hz, 5'''-H), 7.76 (1H, d, J=9 Hz, 5-H), 8.56 (1H, s, 3-NH, exchanged with D_2O), 11.69 (1H, s, 1''-H), 11.73 (1H, br s, 1'''-H, exchanged with D_2O), 12.52 (1H, br, 4-OH, exchanged with D_2O); FAB-MS m/z 681 (M+H), 531, 324, 151.

During the column chromatography, a polar by-product was also isolated by further elution with MeOH - CH_2Cl_2 (1:2), yield 330 mg: MP >150°C (dec); Rf 0 (not moving, CH_2Cl_2 - MeOH - HCOOH, 94:4:2); IR (KBr) cm^{-1} 3400, 2980, 2940, 1700, 1660, 1600; ^1H NMR (300 MHz, DMSO-d_6) δ 1.02, 1.04 (3H, 2s, 5'-CH_3), 1.13, 1.16 (3H, 2s, 5'-CH_3), 2.10 (3H, s, 2'-OAc), 2.14, 2.23, 2.32 (9H, 3s, 5''-CH_3, 8-CH_3, 3'''-CH_3), 3.48 (3H, s, 4'-OCH_3), 3.50 (1H, d, J=9 Hz, 4'-H), 5.37 (1H, m, 2'-H), 5.56 (1H, dd, J=3 and 9 Hz, 3'-H), 5.68, 5.72 (1H, 2d, J=3 Hz, 1'-H), 5.91 (1H, br s, 4''-H), 6.60 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 6.72 (1H, br s, 3''-H), 6.97 (1H, d, J=9 Hz, 6-H), 7.13 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 7.14, 7.15 (1H, 2d, J=3 Hz, 5'''-H, became 2s with D_2O), 7.69 (1H, d, J=9 Hz, 5-H), 11.69 (1H, s, 1''-H), 11.81 (1H, br s, 1'''-H, exchanged with D_2O). This by-product was tentatively assigned as a dimeric carbamate or carbonate based on its ^1H NMR spectrum which indicated the absence of 4-hydroxy proton and 3-amide proton and the presence of duplicated peaks on some of the protons. This polar by-product was also converted to monomeric 2'-hydroxy compound **8b** by treatment with NH_4OH in MeOH.

3-(4-Carbamyl-3-methyl-2-pyrrolylcarbonylamino)-4-hydroxy-8-methyl-7-[3-*O*-(5-methyl-2-pyrrolylcarbonyl)noviosyloxy]coumarin (**8b**)

To a stirred suspension of **29b** (250 mg, 0.368 mmol) in MeOH (15 ml) was added conc NH_4OH (0.6 ml) and the resulting clear solution stirred at room temperature for 7 hours. The solvent was evaporated *in vacuo* to dryness and the residue, suspended in H_2O, was acidified to pH 3 with 3 N HCl. The resulting precipitate was collected to give 168 mg (0.0263 mmol, yield 71.5%) of **8b** as a white crystalline solid: MP 208~210°C (dec); Rf 0.13 (CH_2Cl_2 - MeOH - HCOOH, 94:4:2); UV $\lambda_{max}^{50\%\ EtOH}$ nm (ε) 280 (30,700); IR (KBr) cm^{-1} 3400 (br), 3000, 2940, 1700, 1640, 1600; ^1H NMR (300 MHz, DMSO-d_6) δ 1.09 (3H, s, 5'-CH_3), 1.30 (3H, s, 5'-CH_3), 2.24 (3H, s, 8-CH_3 or 5''-CH_3), 2.26 (3H, s, 8-CH_3 or 5''-CH_3), 2.58 (3H, s, 3'''-CH_3), 3.47 (3H, s, 4'-OCH_3), 3.66 (1H, d, J=10 Hz, 4'-H), 4.17 (1H, br s, 2'-H), 5.48 (1H, br d, J=10 Hz, 3'-H), 5.62 (1H, s, 1'-H), 5.69 (1H, d, J=5 Hz, 2'-OH, exchanged with D_2O), 5.92 (1H, s, 4''-H), 6.78 (1H, br s, 3''-H), 6.8 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 7.21 (1H, d, J=9 Hz, 6-H), 7.30 (1H, br, 4'''-$CONH_2$, exchanged with D_2O), 7.55 (1H, d, 5'''-H), 7.76 (1H, d, J=9 Hz, 5-H), 8.60 (1H, s, 3-NH, exchanged with D_2O), 11.65 (1H, s, 1''-H), 11.74 (1H, br s, 1'''-H, exchanged with D_2O), 12.49 (1H, br, 4-OH, exchanged with D_2O); FAB-MS m/z 639 (M+H).

Anal Calcd for $C_{31}H_{34}N_4O_{11}\cdot 1\frac{1}{2}H_2O$: C 55.91, H 5.61, N 8.42.
Found: C 56.09, H 5.58, N 8.19.

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