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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2434–2439
Bioorganic & Medicinal Chemistry Letters

Solid-phase synthesis of new pyrrolobenzodiazepine–chalcone conjugates: DNA-binding affinity and anticancer activity

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Received 18 January 2008; revised 15 February 2008; accepted 18 February 2008
Available online 5 March 2008

Abstract—A new class of C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine–chalcone conjugates have been prepared by employing a solid-phase synthetic protocol. In this strategy an intramolecular aza-Wittig reductive cyclization approach has been utilized. Interestingly, some of these molecules have shown enhanced DNA-binding affinity and promising anticancer activity on a large number of human cancer cell lines.

Solid-phase combinatorial synthesis has become an important tool in many areas of research; including chemical biology and drug discovery,[1] as the demand for libraries of small organic molecules continue to grow. The development of practical synthetic strategies for building libraries of drug-like molecules is an important aspect for medicinal chemists to enable the cellular functions and to explore new drug candidates.

During the course of our efforts directed towards the development of new anticancer agents as well as in the combinatorial diversification, we were interested in the development of solid-phase synthetic strategy for some new molecules based on PBD linked-chalcone conjugates as potential anticancer agents. Chalcones have received significant attention for their antitumour properties over the last few years, particularly in view of their similar mode of action to the structurally related combretastatin (**1**).[2] The anticancer activity of certain chalcones, known to result in binding to tubulin and prevent it from polymerizing microtubules,[3] is the target for a number of clinical useful anticancer compounds including natural products like paclitaxel, vincristine and colchicine. Furthermore, the ease of preparation of chalcones from substituted benzaldehydes and acetophenones makes them an attractive pharmacophoric scaffold.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a group of potent, naturally occurring, antitumour antibiotics produced by various *Streptomycies* species.[4] These compounds bind selectively in the minor groove of DNA, forming a covalent aminal bond between the electrophilic C11-position of the PBD and the nucleophilic N2-amino group of a guanine base,[5] resulting in their biological activity. A number of naturally occurring synthetic compounds based on this PBD ring system, such as anthramycin, chicamycin, abbeymycin, DC-81 (**2**) and its dimers,[6] have shown varying degrees of DNA-binding affinity and anticancer activity. Recently, we have investigated mixed imine-amide PBD dimers[7] as efficient DNA-binding agents with significant anticancer activity in a number of human cancer cell lines and also developed new hybrids of PBD monomers.[8] A number of PBD conjugates have been designed and synthesized in this laboratory, that have exhibited significant cytotoxic property with increased DNA-binding ability.[9] Further, a variety of PBD based molecules have been synthesized by employing aza-Wittig reductive cyclization strategy in solution-phase[10] as well as on solid-phase.[11] In continuation of these efforts towards the synthesis of structurally modified PBD hybrids and also development of new solid-phase synthetic strategies,[12] we herein report the preparation of a new class of C8-linked PBD–chalcone conjugates (Fig. 1). Moreover,

Keywords: Pyrrolo[2,1-*c*][1,4]benzodiazepines; Chalcones; DNA-binding affinity; Anticancer activity; Aza-Wittig reductive cyclization.
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doi:10.1016/j.bmcl.2008.02.047

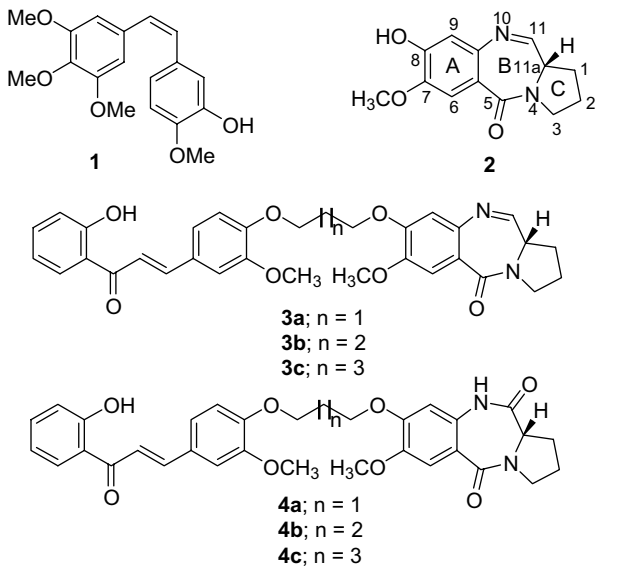


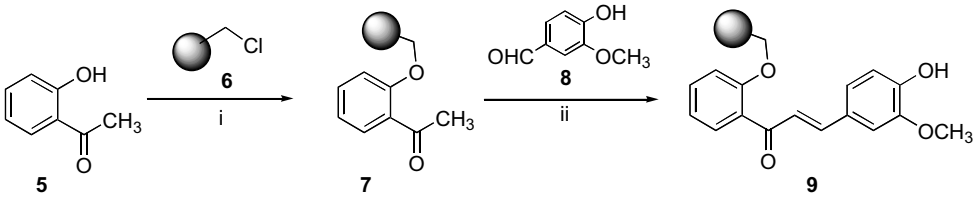
Figure 1. Chemical structures of biologically important combretastatin (**1**), DC-81 (**2**) and PBD–chalcone conjugates (**3a**–**c** and **4a**–**c**).

these molecules have been evaluated for their DNA-binding ability and a representative compound has been investigated for its potential as an anticancer agent.[13]

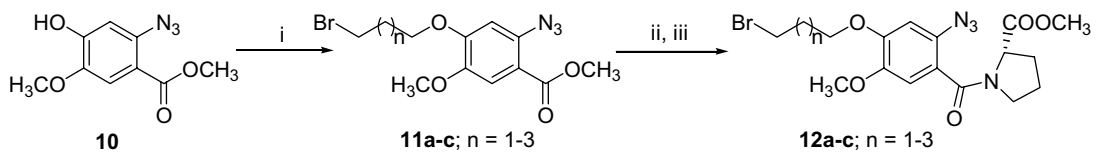
The synthetic pathway started from commercially available 2-hydroxyacetophenone (**5**), which was coupled to commercially available Merrifield resin (**6**, 2.00 mmol/g) with NaH to give resin-bound 2-hydroxyacetophenone (**7**) in excellent yield. This reaction was confirmed by IR spectrum, which showed the keto group absorption at 1739 cm^{-1}. Further, in attempts to condense the resin-bound 2-hydroxyacetophenone **7** with aldehyde, NaOMe (as a 0.5 M solution in MeOH) was found to be the base of choice, the co-solvent with suitable resin swelling properties is necessary to ensure complete conversion to the enone product. Toluene as well as THF gave satisfactory results for this reaction; however, THF is better with respect to consistency of the results for larger scale reactions. Typically 12 equivalents of both aldehyde **8** (vanillin) and NaOMe (0.5 M in MeOH) was added to resin **7** (preswelled in an equal volume of THF) to afford the required resin-bound enone (**9**) as shown in Scheme 1. This reaction was monitored by FT-IR, which shows a strong peak of the carbonyl absorption at 1747 cm^{-1}.

The starting materials **12a**–**c** were prepared from methyl 2-azido-4-hydroxy-5-methoxybenzoate (**10**) which was accomplished by employing the reported method.[14] Etherification of these compounds was performed by using a variety of dibromoalkanes to provide the bromo-substituted azidoesters (**11a**–**c**). These upon hydrolysis in the presence of 2 N LiOH followed by coupling with proline methylester by employing Et_3N afford **12a**–**c** in good yield ranging from 78% to 83% as shown in Scheme 2.

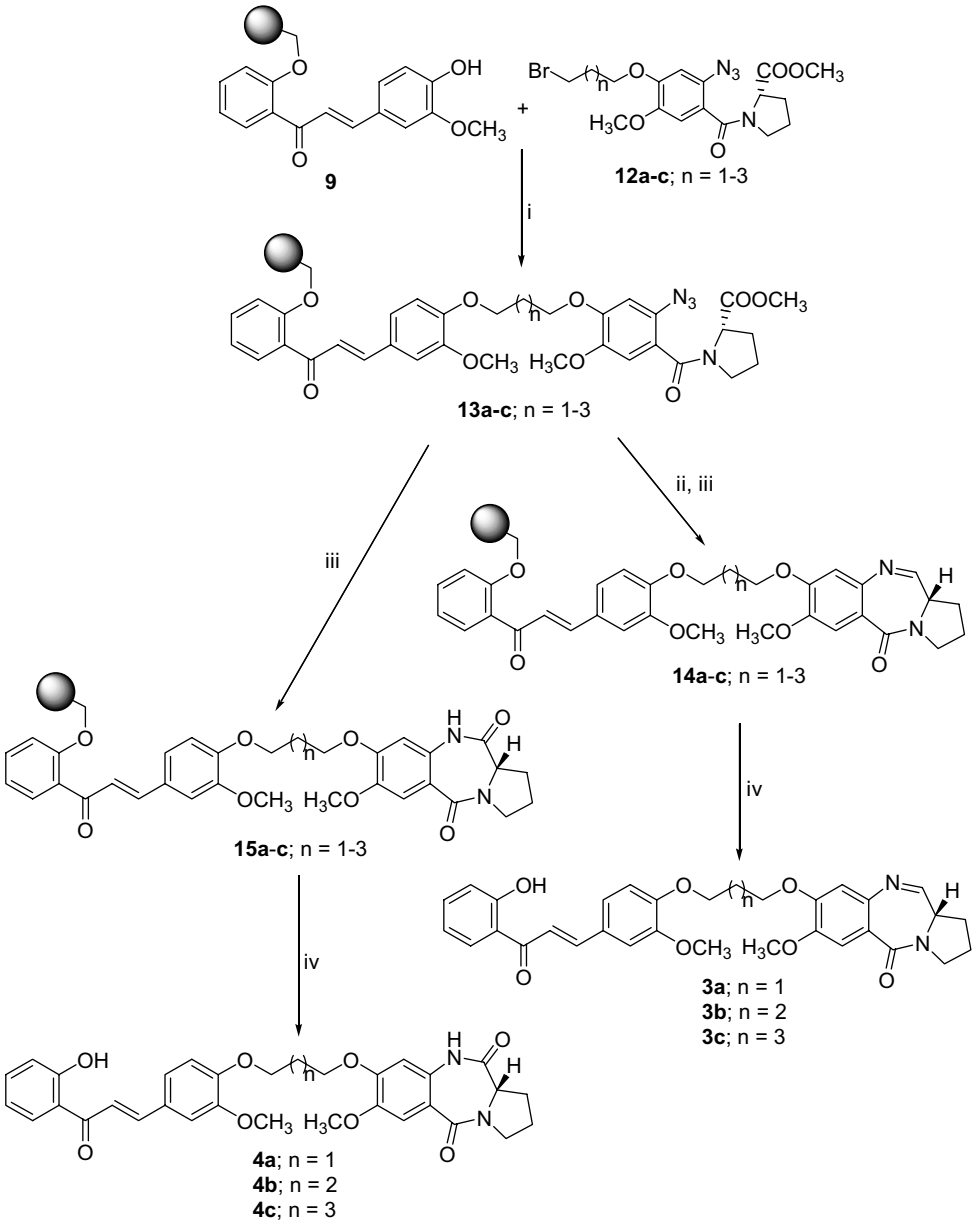
The PBD–chalcone conjugates (**3a**–**c** and **4a**–**c**) were prepared by employing the resin-bound compound **9** and bromo-substituted azidobenzoyl proline methylesters (**12a**–**c**), which were then coupled in the presence of K_2CO_3 to afford the corresponding solid-supported compounds **13a**–**c** as indicated by IR spectra that shows a strong azide stretching at 2110 cm^{-1}. These resin-bound ester functionalities (**13a**–**c**) were reduced selectively with DIBAL-H followed by aza-Wittig reductive cyclization (Staudinger reaction) with triphenyl phosphine (TPP) to produce **14a**–**c**. This step is also confirmed by IR analysis wherein the azido peak is absent. Finally, the resin-bound compounds **14a**–**c** were cleaved by employing TFA/CH_2Cl_2 (1:1) to afford the crude products. This procedure was further repeated once again to ensure the complete cleavage of the product from the resin, which was later purified by column chromatography using (silica gel, 100–200 mesh) ethyl acetate/methanol (9:1) to give **3a**–**c**[15] (yields 65–78%). The resin-bound chalcone–PBD dilactams (**4a**–**c**) have also been prepared by employing a similar procedure. The reduction of resin-bound azido group **15a**–**c** was accomplished by employing TPP and followed by cleav-



Scheme 1. Reagents and conditions: (i) NaH, dry DMF, 50–60 °C, 48 h; (ii) NaOMe/THF/MeOH, rt, 4 days.



Scheme 2. Reagents and conditions: (i) K_2CO_3, dibromoalkanes, DMF, rt, 12 h; (ii) LiOH (2 N), THF/MeOH/H_2O (3:1:1), rt, 2 h; (iii) (a) $(COCl)_2$, dry CH_2Cl_2, 1–3 drops DMF, 0 °C–rt, 2 h; (b) Et_3N, L-proline methylester hydrochloride, THF, 0 °C–rt, 2 h.



Scheme 3. Reagents and conditions: (i) K_2CO_3, dry DMF, 50 °C, 48 h; (ii) DIBAL-H, dry CH_2Cl_2, −78 °C, 2 h; (iii) TPP, dry toluene, rt, 16 h; (iv) TFA/CH_2Cl_2 (1:1), rt, 2 h, 65–78%.

age with TFA/CH_2Cl_2 (1:1) to give **4a**–**c**[15] as shown in Scheme 3.

The DNA-binding ability of these C8-linked chalcone–PBD conjugates was examined by thermal denaturation studies using calf thymus (CT) DNA. These studies show the melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. In this assay, the helix melting temperature changes (ΔT_m) for each compound were studied after 0 and 18 h of incubation at 37 °C. Interestingly, in this study PBD–chalcone conjugates compounds **3a**–**c** have shown melting temperature increases from 1.7 to 8.1 °C (Table 1). The ΔT_m of compound **3a** is 5.0 °C at 0 h, while the melting temperature increases to 8.1 °C upon incubation for 18 h at 37 °C. Compound **3b** elevates the helix melting temperature of CT-DNA by 1.7 °C at 0 h; however, there is not much difference in the melting temperatures after incubation for 18 h (2.1 °C). Whereas for compound **3c** the ΔT_m is 3.1 °C at 0 h while the helix melting temperature increases to 5.0 °C after incubation for 18 h at 37 °C. In the same experiment the naturally occurring DC-81 elevates the helix melting temperature of CT-DNA by 0.7 °C after incubation for 18 h. It is interesting to observe that for both the PBD conjugates **3a** and **3c** the ΔT_m values are significant when the length of alkyl spacer is odd numbered, probably as they have a better fit in the minor groove of DNA.

Similarly, non-covalent DNA-interactive chalcone–PBD dilactam conjugates were evaluated for their

Table 1. Thermal denaturation data for C8-linked PBD–chalcone conjugates (**3a–c** and **4a–c**) with calf thymus (CT) DNA

Compound	[PBD]/[DNA] molar ratio[a]	ΔT_m[b] (°C) after incubation at 37 °C for	
		0 h	18 h
3a	1:5	5.0	8.1
3b	1:5	1.7	2.1
3c	1:5	3.1	5.0
4a	1:5	2.0	3.5
4b	1:5	1.0	1.9
4c	1:5	7.1	9.0
DC-81	1:5	0.3	0.7

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 0.01 (mean value from 10 separate determinations), all T_m values are ± 0.05–0.1 °C.

[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

DNA-binding affinity by thermal denaturation studies using a similar procedure. These conjugates **4a–c** also exhibited significant helix melting temperature values ranging from 1.0 to 9.0 °C. Compounds **4a** and **4b** have shown noticeable DNA-binding affinity of 2.0 and 1.0 °C with calf thymus CT-DNA and there is not much difference in their helix melting temperatures upon incubation at 37 °C for 18 h (3.5 and 1.9 °C). However, ΔT_m of compound **4c** is 7.1 °C at 0 h while the melting temperature increases to 9.0 °C upon incubation for 18 h (Table 1). It is interesting to observe that compound **4c** showed enhanced DNA-binding ability compared to chalcone–PBD imine conjugates (**3a–c**).

Compound **3a** as a representative member of this class that exhibited significant DNA-binding ability has been evaluated for its in vitro activity against the standard sixty human tumour cell lines, derived from nine cancer types (leukaemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer). This compound shows good anticancer potency in a wide spectrum of cell lines causing 50% cell growth inhibition (GI_{50}), and the values range from 0.01 to 0.40 μM as seen from Table 2. Moreover, it exhibits less than 30 nM potency as seen from the GI_{50} values in almost 32 cancer cell lines. Further the cytotoxic potency (LC_{50}) is significant in some selected human cancer cell lines ranging from 0.07 to 0.98 μM. The in vitro cytotoxicity (LC_{50}) for the naturally occurring DC-81 is 0.38 and 0.33 μM in L1210 and PC6 cancer cell lines, respectively.[16] It is interesting to note that the mean graph midpoint values of compound **3a** are $\log_{10}GI_{50}$ (−7.43), $\log_{10}TGI$ (−6.51) and $\log_{10}LC_{50}$ (−5.18).

In conclusion, the synthesis of biologically important DNA-interactive C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine–chalcone conjugates has been demonstrated for the first time. In this process solid-supported synthetic method, aldol condensation and intramolecular aza-Wittig reductive cyclization process have been utilized. These chalcone–PBD conjugates have shown noticeable DNA-binding affinity and potential anticancer activity in a number of human cancer cell lines for a representative compound of this class.

Table 2. In vitro anticancer activity of compound **3a** on 60 human cancer cell lines

Cancer panel/cell line	GI_{50} (μM)	Cancer panel/cell line	GI_{50} (μM)
Leukaemia		*Ovarian*	
CCRF-CEM	0.021	IGROV1	0.018
HL-60(TB)	0.017	OVCAR-3	0.021
K-562	0.026	OVCAR-4	0.061
MOLT-4	0.017	OVCAR-5	0.025
RPMI-8226	0.025	OVCAR-8	0.036
SR	0.012	SK-OV-3	0.406
Non-small cell lung		*Renal*	
A549/ATCC	0.031	786-0	0.025
EKVX	0.204	ACHN	0.047
HOP-62	0.039	CAKI-1	0.025
HOP-92	0.044	RXF 393	0.02
NCI-H226	0.138	SN12C	0.028
NCI-H23	0.039	TK-10	0.041
NCI-H322M	0.307	UO-31	0.105
NCI-H460	0.034		
NCI-H522	0.026	*Prostate*	
		DU-145	0.025
Colon		*Breast*	
COLO 205	0.027	MCF7	0.032
HCC-2998	0.138	NCI/ADR-RES	0.308
HCT-116	0.033	231/ATCC	0.026
HCT-15	0.247	HS 578T	0.023
HT29	0.030	MDA-MB-435	0.019
KM12	0.018	BT-549	0.017
SW-620	0.019	T-47D	0.029
		MDA-MB-468	0.016
CNS			
SF-268	0.024		
SF-539	0.028		
SNB-19	0.029		
SNB-75	0.036		
U251	0.022		
Melanoma			
LOX IMVI	0.018		
MALME-3M	0.037		
M14	0.046		
SK-MEL-2	0.067		
SK-MEL-28	0.020		
SK-MEL-5	0.024		
UACC-257	0.112		
UACC-62	0.021		

Acknowledgments

We thank National Cancer Institute (NCI), Maryland, for providing data on anticancer assay in human cancer cell lines and the authors N.S., S.P.R., Ch.R.R., N.M.K., K.L.R. and V.D. are grateful to CSIR, New Delhi, for the award of Research fellowships.

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15. Typical procedure for compound **3a**: To a suspension of Merrifield resin (**6**) (2000 mg, 2.00 mmol/g, 1% cross-linked) in DMF (30 mL), 2-hydroxyacetophenone (**5**, 554 mg, 4.0 mmol) was added followed by NaH (276 mg, 6.0 mmol) at 0 °C, then heated to 50 °C and stirred at the same temperature for 48 h. Further, the reaction mixture was cooled to room temperature, quenched with ice-cold water, and then filtered, washed with DMF (3× 10 mL), H_2O (3× 10 mL), CH_3OH (3× 10 mL), CH_2Cl_2 (3× 10 mL) and then dried under vacuum to give resin-bound 2-hydroxyacetophenone (**7**). Later a solution of NaOMe in MeOH (31.30 mL of a 0.5 M solution in MeOH, 15.6 mmol) was added to a mixture of resin-bound 2-hydroxyacetophenone (**7**, 2.34 g) and 4-hydroxy-3-methoxybenzaldehyde (**8**, 2.63 g, 17.38 mmol) in anhydrous THF (30 mL). Then the flask was capped and placed on an orbital shaker for 4 days. The reaction mixture was filtered and washed sequentially with the following solvents THF (3× 40 mL), MeOH (3× 40 mL), THF (3× 40 mL), MeOH (3× 40 mL) and finally washed with THF (3× 40 mL). After which the resin was dried on a Buchner funnel to give **9**. Later to a suspension of resin (**9**, 0.886 g, 1.7 mmol/g) in dry DMF (15 mL), K_2CO_3 (1.17 g, 8.5 mmol) and **12a** (1.49 g, 3.4 mmol) were added and the reaction mixture was stirred at 50 °C for 48 h to afford **13a**. Then the reaction mixture was brought to room temperature and filtered, washed sequentially with the following solvents DMF (3× 15 mL), DMF/water (8:2, 3× 15 mL), DMF (3× 15 mL), THF (3× 15 mL) and finally washed with CH_2Cl_2 (3× 15 mL) then dried. Further, to a suspension of resin (**13a**, 0.440 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was added DIBAL-H (2.28 mL of a 1 M solution in hexane, 2.28 mmol) drop wise at −78 °C under nitrogen atmosphere, and the reaction mixture was stirred at the same temperature for 2 h. Then the reaction was quenched by the addition of 10 mL of 0.5% HCl, filtered off and rinsed with hexane, water, THF, CH_2Cl_2 and dried in vacuo for 2 h. The derivatized resin (0.382 g, 0.57 mmol) was taken in round bottom flask, dry toluene (20 mL) and TPP (0.760 g, 2.85 mmol) was added and allowed to stir for 5 h at room temperature to afford reductive cyclized resin-bound product **14a**. This resin was filtered and washed with toluene (2× 25 mL), CH_2Cl_2 (2× 25 mL), ether (2× 25 mL) and then dried. Finally, the resin (**14a**, 0.345 g) was cleaved by employing TFA/CH_2Cl_2 (1:1, 10 mL) to afford the crude product **3a**. This procedure was repeated once again to ensure the complete cleavage of the product from the resin. The filtrate was saturated with aqueous $NaHCO_3$ solution, and then extracted with ethyl acetate; the organic layer was separated, dried over Na_2SO_4 and evaporated in vacuo to afford the crude product. This upon purification by column chromatography using (silica gel, 100–200 mesh) ethyl acetate/methanol (9:1) as eluent gave compound (**3a**, 25 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.94 (s, 1H); 7.92–7.95 (d, 1H, J = 7.79 Hz); 7.65–7.67 (t, 1H, J = 3.12, 3.90 Hz); 7.47–7.54 (m, 2H); 7.16 (s, 1H); 6.93–7.07 (m, 4H); 6.86 (s, 1H); 5.39–5.44 (dd, 1H, J = 3.12, 10.13 Hz); 4.20–4.34 (m, 4H); 3.93 (s, 3H); 3.88 (s, 3H); 3.53–3.84 (m, 2H); 2.28–2.45 (m, 4H); 2.02–2.08 (m, 2H); 1.71 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.0; 22.5; 24.1; 29.3; 31.5; 44.5; 46.6; 53.6; 56.0; 65.3; 79.4; 112.8; 117.8; 118.5; 121.5; 123.3; 126.9; 127.7; 129.5; 131.5; 136.1; 140.7; 145.6; 147.8; 149.7; 150.7; 151.2; 161.5; 162.3; 164.5; 192.0; 193.5; FABMS: m/z 557 [M^++H]; HRMS calcd for $C_{32}H_{32}N_2O_7$ 556.3768, found 556.3772.
Compound **3b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.94 (s, 1H); 7.91–7.96 (m, 3H); 7.66–7.67 (d, 1H, J = 4.30 Hz); 7.47–7.54 (m, 2H); 6.91–7.07 (m, 5H); 6.82 (s, 1H); 5.40–5.44 (dd, 1H, J = 2.15, 10.77 Hz); 4.09–4.22 (m, 4H); 3.93 (s, 3H); 3.89 (s, 3H); 3.55–3.85 (m, 2H); 2.30–2.36 (m, 2H); 2.01–2.12 (m, 6H); FABMS: m/z 571 [M^++H]. HRMS calcd for $C_{33}H_{34}N_2O_7$ 570.4033, found 570.4028.

Compound **3c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.81 (s, 1H); 7.81–7.90 (m, 3H); 7.60–7.61 (d, 1H, J = 3.90 Hz); 7.42–7.49 (m, 2H); 6.83–7.04 (m, 5H); 6.72 (s, 1H); 5.35–5.39 (dd, 1H, J = 2.34, 10.13 Hz); 4.02–4.12 (m, 4H); 3.92 (s, 3H); 3.91 (s, 3H); 3.52–3.85 (m, 2H); 2.27–2.34 (m, 2H); 1.89–2.08 (m, 6H); 1.66–1.75 (m, 2H); FABMS: *m/z* 585 [M^++H]; HRMS calcd for $C_{34}H_{36}N_2O_7$ 584.4293, found 584.4298.

Compound **4a** was prepared according to the procedure described in the earlier preparation of **3a**. The resin-bound dilactam (**15a**, 0.325 g) was added by employing TFA/CH_2Cl_2 (1:1, 10 mL) to afford the product (**4a**, 20 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.79 (s, 1H); 8.74 (br s, 1H); 7.77–7.90 (m, 3H); 7.36–7.50 (m, 2H); 7.15–7.19 (m, 1H); 6.85–7.04 (m, 4H); 6.54 (s, 1H); 5.33–5.38 (dd, 1H, J = 3.02, 10.57 Hz); 4.10–4.20 (m, 4H); 3.92 (s, 3H); 3.87 (s, 3H); 3.50–3.75 (m, 2H); 2.20–2.33 (m, 2H); 1.85–2.02 (m, 4H); FABMS: *m/z* 573 [M^++H]; HRMS calcd for $C_{32}H_{32}N_2O_8$ 572.3762, found 572.3768.

Compound **4b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.82 (s, 1H); 8.88 (br s, 1H); 7.78–7.92 (m, 3H); 7.38–7.56 (m, 2H); 7.19–7.25 (m, 1H); 6.88–7.10 (m, 4H); 6.56 (s, 1H); 5.31–5.35 (dd, 1H, J = 3.12, 10.26 Hz); 4.11–4.22 (m, 4H); 3.96 (s, 3H); 3.89 (s, 3H); 3.51–3.80 (m, 2H); 2.21–2.36 (m, 2H); 1.84–2.12 (m, 6H); FABMS: *m/z* 587 [M^++H]; HRMS calcd for $C_{33}H_{34}N_2O_8$ 586.4027, found 586.4039.

Compound **4c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.98 (s, 1H); 8.91 (br s, 1H); 7.85–7.98 (m, 2H); 7.41–7.59 (m, 3H); 6.91–7.12 (m, 5H); 6.42 (s, 1H); 5.40–5.45 (dd, 1H, J = 3.11, 10.68 Hz); 4.09–4.19 (m, 4H); 3.96 (s, 3H); 3.88 (s, 3H); 3.55–3.79 (m, 2H); 2.21–2.25 (m, 4H); 1.95–2.12 (m, 6H); 1.65–1.75 (m, 2H); FABMS: *m/z* 601 [M^++H]. HRMS calcd for $C_{34}H_{36}N_2O_8$ 600.4292, found 600.4298.

16. Bose, D. S.; Thompson, A. S.; Smellie, M.; Berardini, M. D.; Hartley, J. A.; Jenkins, T. C.; Neidle, S.; Thurston, D. E. *J. Chem. Soc. Chem. Commun.* **1992**, *9*, 1518.