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Design, Synthesis, and Biological Evaluation of Caprolactam-Modified Bengamide Analogues

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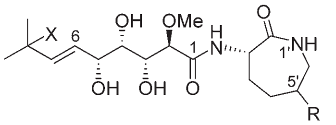
The sponge-derived bengamides, first isolated by Crews and co-workers in 1986,[1] have a unique molecular structure and a broad array of biological activities that include antitumor, antibiotic, and anthelmintic properties.[2] Because of their striking and attractive antitumor properties, these molecules have been the focus of many studies on their synthetic[3] and biological[4] aspects. Bengamide B (Figure 1), the most promising of this family,[5] and its 5′-position-derived ester analogues, were investigated fully by Kinder et al.[6] One bengamide analogue, LAF389[7] (Figure 1), has been used in a clinical trial; however, the poor pharmacokinetic properties and unclear side effects of LAF389, which appeared early in the trial, have prevented its further development.[8]



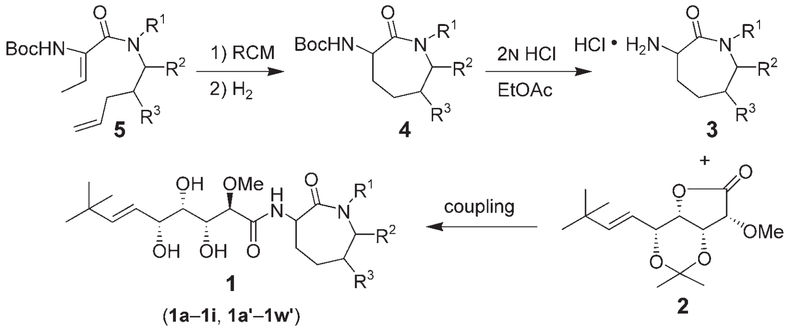
bengamide B R= OCOC$_{13}$H$_{27}$, 5′ *S*, X = H

LAF389 R= OCO-*c*Hex, 5′ *R*, X= CH$_3$

Figure 1. Bengamide B and LAF389.

Until now, the structural modification of bengamides has focused mainly on improving their water solubility and ease of synthesis. Side chain modification has proven successful, and isopropyl replacement by *tert*-butyl has significantly simplified the synthesis of their analogues. However, these modifications have not yet shown clear structure–activity relationships (SAR) compared with other studies of side chain optimization. There have been few reports on diverse modifications of the caprolactam unit until recently, possibly because of the lack of a simple and flexible method to synthesize functionalized caprolactams. Following our successful construction methodology for the ring-closing metathesis (RCM) reaction of α-aminoacrylamides to substituted aminocaprolactams,[9] we now present a series of bengamide analogues modified at the 5′-, 6′-, and 7′-positions of the caprolactam subunit and their antitumor activity on MDA-MB-435 human breast carcinoma cells. The chemistry used to prepare these analogues is illustrated in Scheme 1.

Bengamide analogues (**1a**–**1i**) were synthesized from a known lactone fragment **2**[7] and substituted caprolactam **3** by a coupling reaction. The key functionalized caprolactam **3** was obtained through hydrogenation and deprotection of the cyclization products prepared from **5** using the RCM strategy reported previously.[9]



Scheme 1. Design of 5′-, 6′-, and 7′-substituted bengamide analogues (respectively specified positions R^1, R^2, and R^3; see Table 1).

5′-Substituted caprolactams were synthesized from alkene–amide RCM products through a multistep conversion (**8**–**11**), or through derivation of compound **9** (**12** and **13**), as illustrated in Scheme 2. Simple hydrogenation of cyclized products **6** and **7** gave key intermediates **8** and **9**, which were deprotected to afford compounds **10** and **11**. Caprolactams **12** and **13**[10] were obtained from compound **9** through hydrolysis, mesylation, azidation, hydrogenation, acylation, and deprotection.

The simple N-substituted bengamide analogues (**1a**′–**1w**′) were prepared from diverse N-substituted caprolactams, which were synthesized by using a similar procedure, as outlined in Scheme 3 and Scheme 4, to give simple N-alkylating products **18a**–**18c**, **18d**, **18p**, **18u** (Scheme 3) and **18i**–**18k**, **18l**–**18o** (Scheme 4). Compounds **18e** (enantiomer of **18d**) and **18o** were prepared from the pure enantiomer **17**′, and other intermediates were obtained from racemic compound **17.** After de-

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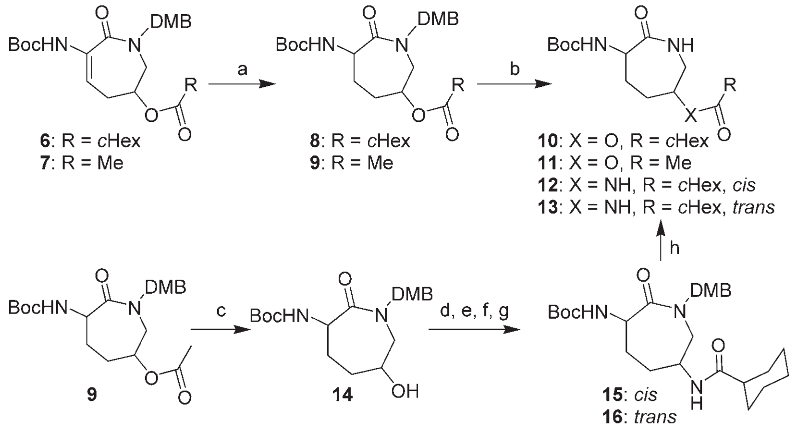
protection of key intermediates **8**–**13** and **18 a**–**18 w**, all bengamide analogues **1 a**–**1 i**, **1 a**′–**1 w**′ were finally obtained through coupling of substituted caprolactams and lactone as reported[6,7] in 25–70 % yield, as shown in Scheme 1.

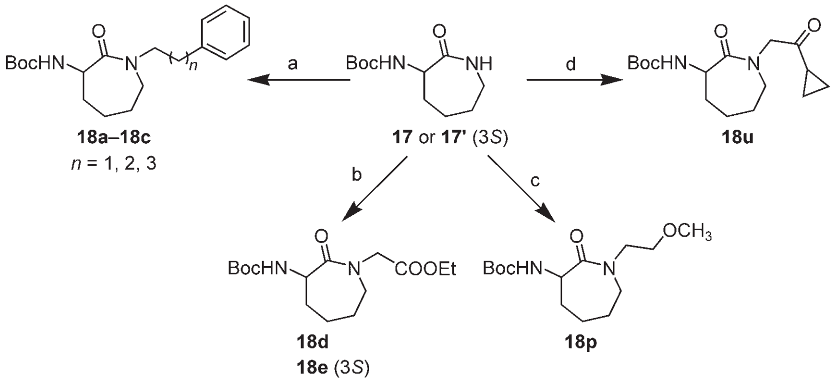
All these compounds were tested on MDA-MB-435 human breast carcinoma cells for their antitumor activity (Table 1). Compound **1 a**, a diastereomeric mixture of LAF389, was used as a positive control, and most of the other bengamide analogues were tested as a mixture, except for those that could be separated easily as a diastereomer in an earlier stage of the synthesis. As can be seen from Table 1, **1 a** displayed significant activity, with an IC_{50} value of 40 nм in our assay system; this is consistent with the activity reported for the pure stereoisomer of LAF389.

From the reported data on LAF389,[8] we deduced that the instability of the 5′-ester may contribute to the rapid metabolism and side effects of LAF389, and we replaced the ester with a more stable amide bond. However, the result was disappointing in that the antitumor activity of both the *cis* (**1 c**) and *trans* (**1 d**) compounds decreased markedly. A diverse modification on this position was studied systematically at Novartis, and several in vitro potent and water-soluble compounds have been discovered, including LAF389, mentioned above. Although the in vivo potency did not differ significantly, we speculate that modification at the other positions may produce more potent compounds.

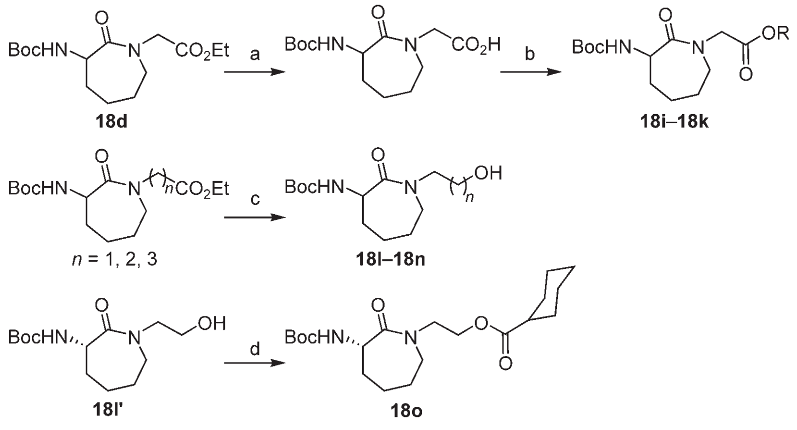
Compound **1 i**, substituted at the 5′- and 6′-positions, is the first type of compound to confirm this speculation. After cycloalkyl substitution at the 6′-position, the activity of **1 i** increased by 4.5-fold relative to



Scheme 2. Synthesis of 5′-substituted caprolactams. Reagents and conditions: a) H_2 (100 kPa), $Pd(OH)_2/C$ (20 wt %), quant.; b) CAN, CH_3CN/H_2O, 45 % for **10**; c) K_2CO_3, MeOH, H_2O, 95 %; d) Et_3N, MsCl, CH_2Cl_2, 0 °C, 97 %; e) NaN_3, DMF, 80 °C, *cis/trans* = 2.1:1, 73 %; f) H_2 (100 kPa), Pd/C (5 %), 99 %; g) $C_6H_{11}COCl$, py, CH_2Cl_2, 56 % for **15**, 58 % for **16**; h) Na/NH_3, THF, 57 % for **12**, 67 % for **13**. CAN = ceric ammonium nitrate, DMF = *N*,*N*-dimethylformamide.

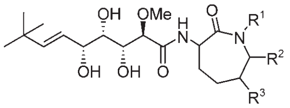


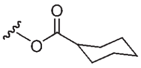
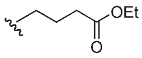
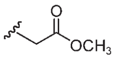
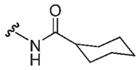
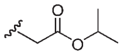
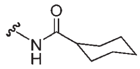
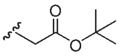
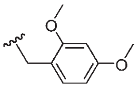
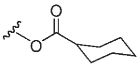
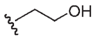
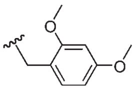
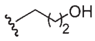
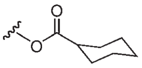
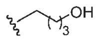
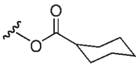
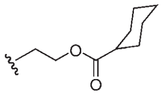
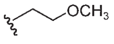
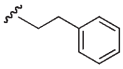
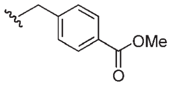
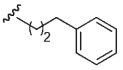
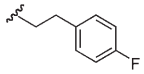
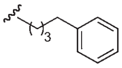
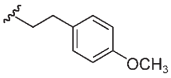
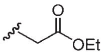
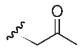
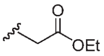
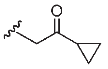
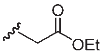
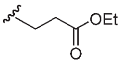
Scheme 3. Synthesis of N-substituted caprolactams. Reagents and conditions: a) NaH, DMF, 0 °C→RT, 1) $Ph(CH_2)_2OTs$, 70 % for **18 a**, 2) $Ph(CH_2)_3OTs$, 80 % for **18 b**, 3) $Ph(CH_2)_4OTs$, 50 % for **18 c**; b) LiHMDS, $BrCH_2COOEt$, 80 % for **18 d**; c) NaH, DMF, $BrCH_2CH_2OCH_3$, RT, 81 %; d) NaH, DMSO, 2-bromo-1-cyclopropylethanone, RT, 62 %. LiHMDS = lithium hexamethyldisilazanide.



Scheme 4. Synthesis from derivatization of N-substituted caprolactam intermediates. Reagents and conditions: a) NaOH, H_2O, THF, RT, 90 %; b) EDC, HOBt, ROH (specified R group, see Table 1, **1 i**′–**1 k**′), RT, 60–83 % for **18 i**–**18 k**; c) $LiBH_4$, THF/EtOH; d) CyCOOH, EDC, DMAP, CH_2Cl_2, 75 %. DMAP = 4-dimethylaminopyridine, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole.

Table 1. Structures and MDA-MB-435 human breast carcinoma in vitro activity of selected bengamide analogues modified at positions 5′, 6′, and 7′.



Compd	R^1	R^2	R^3	IC$_{50}$ [μM][a]	Compd	R^1 (R^2, R^3 = H)	IC$_{50}$ [μM][a]
1a	H	H		0.040 ± 0.002 (1)[b]	**1h′**		1.120 ± 0.240
1b	H	H	OAc	2.092 ± 0.231	**1i′**		0.25 ± 0.021
1c (2′, 5′ *cis*)	H	H		2.393 ± 0.382	**1j′**		0.424 ± 0.017
1d (2′, 5′ *trans*)	H	H		2.013 ± 0.180	**1k′**		0.275 ± 0.005
1e		H		0.712 ± 0.016	**1 L′**		0.202 ± 0.005
1f		H	OAc	0.364 ± 0.059	**1m′**		0.424 ± 0.068
1g (more polar)[c]	OH	H		0.078 ± 0.010	**1n′**		0.358 ± 0.071
1h (less polar)[c]	OH	H		0.312 ± 0.014	**1o′** (2′*S*)		0.017 ± 0.008 (10)
1i	H		OAc	0.466 ± 0.033	**1p′**		0.396 ± 0.046
1a′		H	H	0.287 ± 0.039	**1q′**		0.236 ± 0.064
1b′		H	H	1.306 ± 0.476	**1r′**		0.557 ± 0.129
1c′		H	H	0.275 ± 0.028	**1s′**		0.269 ± 0.041
1d′ (2′*S*+2′*R*)		H	H	0.305 ± 0.017	**1t′**		0.276 ± 0.029
1e′ (2′*S*)		H	H	0.141 ± 0.009 (20)	**1u′**		1.286 ± 0.226
1f′ (2′*R*)		H	H	1.617 ± 0.274	**1v′**	Me	0.626 ± 0.234
1g′		H	H	2.113 ± 0.532	**1w′**		0.781 ± 0.160
doxorubicin				0.281 ± 0.035			

[a] Values represent the average ± SEM; percent net growth = [(cell + drug) $A_{550/690}$ − initial $A_{550/690}$/(cell + drug vehicle DMSO) $A_{550/690}$ − initial $A_{550/690}$] × 100; values in parentheses indicate solubility in H_2O [mg mL^{-1}]. [b] For **1a**, solubility refers to its diastereomer LAF389. [c] The configuration of these two compounds were not determined, but they were separated by column chromatography.

1b. This preliminary result indicates that proper substitution at the 6′-position is tolerated. Among the N-substituted caprolactam bengamide analogues, **1f** displayed activity with an IC$_{50}$ value 5.7 times that of **1b**. At the same time, compared with compound **1e** (IC$_{50}$ = 0.71 μM), compounds **1g** and **1h** showed potent activity, with IC$_{50}$ values of 78 nM and 0.31 μM, respectively. These data indicate that N-substitution on caprolactam greatly influences the antitumor activity.

Simple N-substituted bengamides are very interesting because previous reports including research at Novartis[6] have indicated that this position does not tolerate further modification except with H and Me groups. However, our research reveals that SAR at this position differs slightly from those reported. We found that diverse functional group introduction at the 7′-position may greatly influence the antitumor activity, which may present a new starting point for further modification.

Based on the speculations mentioned above and the simplified synthetic path of bengamide analogues, we introduced limited diverse functional groups on the amide nitrogen atom, including aryl, ester, hydroxy, ether, and keto groups. All functional groups displayed a certain amount of activity, with IC$_{50}$ values ranging from 0.017 μM to 2.110 μM. Interestingly, by comparing the most potent analogue **1 o**′ with **1 a**, one can find that transfer of the cyclohexylcarbonyloxy group from the 5′-position of **1 a** to the amide N atom, tethered with a two-methylene-unit linker, forms a more potent and simpler analogue **1 o**′. This compound has an IC$_{50}$ value of 17 nM and solubility in H$_2$O of 10 mg mL^{-1}, values that are more advantageous than the values of **1 a**, the IC$_{50}$ value of which is 40 nM and solubility is 1 mg mL^{-1}. Another potent compound, **1 e**′, has an activity of 0.141 μM and a solubility in H$_2$O of 20 mg mL^{-1}. Investigation of the antitumor activity of *N*-acetic ethyl ester derived bengamide analogues **1 d**′, **1 e**′, and **1 f**′ indicates that the 2′*S* epimer **1 e**′ displays the best result with an IC$_{50}$ value of 0.141 μM. Another epimer (**1 f**′, 2′*R*) has a low IC$_{50}$ value of 1.617 μM, and a diastereomeric mixture of these two epimers **1 d**′ produced only modest activity (IC$_{50}$ = 0.305 μM). Compounds **1 g** and **1 h** also show that configurational differences at the 2′-position decreased potency by a factor of four (IC$_{50}$ = 0.078 μM and 0.312 μM, respectively).

From these results, we can clarify some primary SAR for simple N-substituted bengamide analogues. In the case of esters and alcohols, antitumor activity decreased first but then increased as the chain length of the R group increased (see **1 d**′, **1 g**′, **1 h**′, and **1 l**′–**1 n**′), and the two-carbon chain length gave the best result. In addition, for esters, increasing the substituent size at the terminal position of the R group seemed to have little effect on activity (see **1 i**′ and **1 k**′), but this effect is clear in the case of ketones (**1 t**′ and **1 u**′). For aryl groups, the influence of alkyl chain length and substitutional effects were still not clear (**1 a**′–**1 c**′ and **1 q**′–**1 s**′). For simple alkyl functional groups, the increased length led to slightly decreased activity (**1 v**′ and **1 w**′), and in both cases, inferior activity relative to other functional groups was observed.

In summary, the present work provides a new strategy for research on bengamide analogues. We demonstrated for the first time that 5′-, 6′-, and 7′-position-substituted bengamide analogues display activity in a general way and that the 7′-position, in particular, accommodates diverse substitution. Derivation from this position produced a new potent compound, which is a more potent and water-soluble analogue, **1 o**′ (IC$_{50}$ = 17 nM, water solubility: 10 mg mL^{-1}), than LAF389. The synthesis of these potent compounds may present a simpler new method to produce natural product bengamide-like compounds with potent antitumor activity. More detailed SAR studies focusing on this type of compound and their effects in vitro and in vivo are in progress.

Experimental Section

General procedure for the synthesis of compounds 18 a–18 w: a solution of compound **17** (1 g, 4.3 mmol) in THF (8 mL) was added to a suspension of NaH (200.0 mg, 4.8 mmol) in THF (10 mL) at room temperature. This mixture was stirred for 1 h, and a solution of phenethyl-4-methylbenzenesulfonate (1.3 g, 4.8 mmol) in THF (8 mL) was added. After additional stirring for 2 h the reaction mixture was cooled to 0 °C, treated with a saturated solution of NH$_4$Cl, and extracted with AcOEt. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography to give **18 a** (0.45 g, 41 %). ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 1.45$ (s, 9 H), 1.74–2.04 (m, 6 H), 2.84 ((m, 2 H), 3.08 (dd, $J = 15.6$, 4.8 Hz, 1 H), 3.45 (m, 2 H), 3.80 (m, 1 H), 4.34 (m, 1 H), 6.02 (d, $J = 5.4$ Hz, 1 H), 7.20–7.33 ppm (m, 5 H).

Following reported procedures,*[6, 7] *bengamide analogues 1 a–1 i, 1 a′–1 w′ were synthesized:

1 g: ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 7.99$ (d, $J = 6.3$ Hz, 1 H), 5.83 (d, $J = 15.8$ Hz, 1 H), 5.41 (dd, $J = 15.9$, 7.2 Hz, 1 H), 4.92 (m, 1 H), 4.59 (m, 1 H), 4.22 (t, $J = 6.6$ Hz, 1 H), 4.12 (m, 1 H), 3.83–3.75 (m, 2 H), 3.62 (d, $J = 4.2$ Hz, 1 H), 3.55 (s, 3 H), 3.47 (dd, $J = 15.9$, 5.4 Hz, 1 H), 3.37 (m, 1 H), 3.04 (m, 1 H), 2.29 (m, 1 H), 2.13 (m, 1 H), 2.02–1.62 (m, 8 H), 1.41–1.22 (m, 5 H), 1.02 ppm (s, 9 H); ^{13}C NMR (CDCl$_3$, 75 MHz): $\delta = 175.2$, 172.0, 166.8, 146.1, 123.3, 81.6, 74.8, 72.8, 72.6, 65.2, 60.0, 51.4, 50.5, 43.3, 33.3, 32.1, 32.0, 31.5, 30.5, 29.9, 29.6, 29.1, 29.0, 25.9, 25.7, 25.6, 25.5 ppm; HRMS (ESI): *m/z*: 537.2803 [M+Na$^+$], $C_{25}H_{42}N_2O_9Na$ requires 537.2788.

1 e′: ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 8.09$ (d, $J = 6.0$ Hz, 1 H), 5.82 (d, $J = 15.6$ Hz, 1 H), 5.41 (dd, $J = 15.6$, 7.2 Hz, 1 H), 4.66 (dd, $J = 10.1$, 6.6 Hz, 1 H), 4.38 (br s, 1 H), 4.30–4.04 (m, 5 H), 3.82–3.74 (m, 2 H), 3.72 (br d, $J = 3.9$ Hz, 1 H), 3.59 (m, 1 H), 3.53 (s, 3 H), 3.23 (dd, $J = 15.3$, 4.8 Hz, 2 H), 2.03 (m, 2 H), 1.80–1.66 (m, 5 H), 1.28 (t, $J = 7.2$ Hz, 3 H), 1.02 ppm (s, 9 H); ^{13}C NMR (CDCl$_3$, 75 MHz): $\delta = 173.1$, 172.3, 169.7, 145.9, 123.3, 80.7, 74.8, 73.1, 72.5, 61.7, 60.3, 52.4, 51.3, 51.0, 33.2, 31.4, 29.6, 28.0, 27.1, 14.4 ppm; HRMS (ESI): *m/z*: 481.2478 [M+Na$^+$], $C_{22}H_{38}N_2O_8Na$ requires 481.2526.

1 o′: ^{1}H NMR (CDCl$_3$, 300 MHz): $\delta = 8.08$ (d, $J = 6.0$ Hz, 1 H), 5.81 (d, $J = 15.6$ Hz, 1 H), 5.43 (dd, $J = 15.6$, 7.2 Hz, 1 H), 4.60 (dd, $J = 10.7$, 6.3 Hz, 1 H), 4.36 (m, 1 H), 4.23–4.16 (m, 3 H), 3.78 (m, 2 H), 3.66 (br t, $J = 5.7$ Hz, 1 H), 3.57 (m, 1 H), 3.52 (s, 1 H), 3.33 (dd, $J = 15.3$, 4.8 Hz, 2 H), 2.27 (m, 1 H), 2.20–1.60 (m, 8 H), 1.59–1.20 (m, 6 H), 1.00 ppm (s, 9 H); ^{13}C NMR (CDCl$_3$, 75 MHz): $\delta = 176.0$, 172.7, 172.1, 145.7, 123.4, 81.0, 74.7, 73.0, 72.6, 62.3, 60.1, 52.3, 50.1, 48.3, 43.3, 33.2, 31.5, 29.6, 29.2, 29.1, 27.8, 27.7, 25.9, 25.6 ppm; HRMS (ESI): *m/z*: 549.3127 [M+Na$^+$], $C_{27}H_{46}N_2O_8Na$ requires 549.3152.

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[10] *cis* and *trans* isomers were separated by column chromatography after the azidation step, which gave 73% overall yield of both isomers; see Scheme 2e.

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