Efficient Syntheses of Naphthoquinone-Dipeptides

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Abstract: Naphthoquinone-dipeptides are synthesized in 76–89% yield from naphthoquinone–amino acid conjugates by *N*-acylbenzotriazole methodology in aqueous media at 20 °C.

Key words: quinones, amino acids, peptides, coupling, conjugation

In the biochemistry of living cells, quinones play many vital roles including respiration, photosynthesis and cellular defense against bacteria, fungi, and parasites.[1] Quinone derivatives are used as medicines for bacterial and fungal diseases and can exhibit potent antimalarial capacity.[2] Many quinones, some naturally occurring (Figure 1), are antitumor agents and those approved for clinical use include: menadione (**1**), anthracycline-glycosides [doxorubicin (**2**), daunorubicin, and aclacinomycin A], benzoquinone derivatives [mitomycin C (**3**), carbazilquinone, and diaziquone] and more complex quinones [mitoxantrone (**4**), streptonigrin, and actynomicin D].[3–5] The cell cytotoxicity of quinones is due to their ability to (a) undergo a reversible one-electron reduction to a semiquinone radical anion, and (b) to associate and intercalate with DNA duplexes, thus impairing appropriate template function and nucleic acid synthesis.[6]

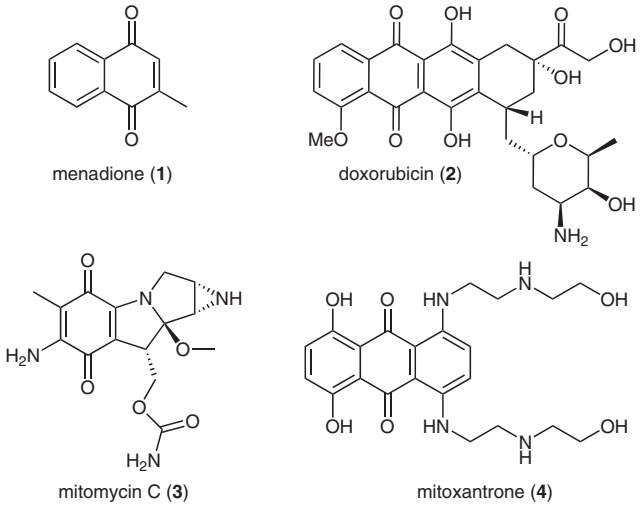

Figure 1 Naturally occurring antitumor quinones

Some human tumors are hormone-dependent and contain the corresponding hormone receptors; some of these, including luteinizing hormone-releasing hormone (LH-RH),[7] somatostatin,[8] bombesin,[9] vasoactive intestinal peptide[10] and growth factors, including epidermal[11] and insulin-like substances,[12] have been detected in prostate, breast, pancreas, ovary, lung, colon, and brain tumors.[13] In view of the abundance of tumors bearing LH-RH receptors, related target chemotherapy has gained considerable attention. Analogues of LH-RH, agonists and antagonists, when conjugated to cytotoxic compounds such as alkylated nitrogen mustard,[14] anticancer antibiotics and quinone derivatives,[15] have exhibited diverse specific binding affinities towards LH-RH receptors. The cytotoxicity of peptide–drug conjugates can be markedly augumented in vitro, far beyond that of the drug component alone.[16]

Preliminary findings indicate that quinonyl-amino acids incorporated into a biological active peptide showed cytotoxic and anticancer activity,[8] and this aroused our interest in the synthesis of quinone-amino acid dipeptides. Several such peptides with quinone moieties attached through the ε-amino side chain of a D-lysine residue possess cytotoxic activity against human breast and prostate cancer cell lines.[15,17]

Bittner and coworkers reported the linking of several free and blocked amino acids to a quinone moiety, and studied free radical generation via chemical and enzymatic methods.[2a,6,7,17,18] Tandon and Maurya have recently reported the unprecedented nucleophilic substitution of 1,4-quinones with amino acids in aqueous suspension.[19] *N*-(1,4-Naphthoquinonyl)glycyl-glycine (**8a**) and *N*-(2-chloro-1,4-naphthoquinonyl)glycyl-glycine (**8b**) were synthesized[17] by reaction of glycyl-glycine with 1,4-naphthoquinone (**5a**) and 2,3-dichloro-1,4-naphthoquinone (**5b**), respectively, in aqueous ethanol during 24–48 hours at room temperature. The initially formed hydroquinone conjugates (**7a** and **7b**) were spontaneously oxidized by excess of 1,4-naphthoquinone to the naphthoquinone–dipeptide conjugates (**8a** and **8b**; 63% and 48%, respectively; Scheme 1).

We felt that, in view of the potential clinical significance of quinone-bearing peptides in targeted chemotherapy, it was important to increase the arsenal of related natural naphthoquinonyl–amino acids, to synthesize them in good yields and to study their spectral properties. Herein, we report an efficient *N*-acylbenzotriazole-mediated preparation of naphthoquinone–dipeptides in yields of 76–89% starting from naphthoquinone-α-amino acid conjugates in aqueous media at 20 °C.

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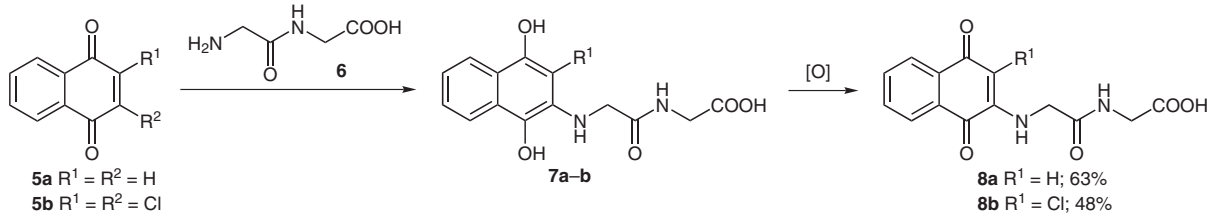
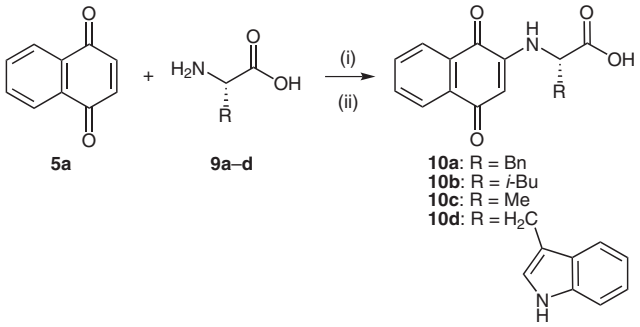


Scheme 1

Naphthoquinone–amino acid conjugates **10a–d** were synthesized starting from naphthoquinone (**5a**) and L-amino acids **9a–d** by a modified literature procedure[20] in aqueous ethanol at room temperature for 10–12 hours in the presence of triethylamine. The reaction mixtures were purified by column chromatography to first yield naphthoquinone–amino acid triethyl ammonium salts, which, after washing with aqueous hydrochloric acid solution, yielded the expected naphthoquinone–amino acid conjugates **10a–d** in 58–79% yields (Scheme 2, Table 1).



Scheme 2 *Reagents and conditions*: (i) Et_3N, EtOH–H_2O, r.t., 12 h; (ii) HCl (10% solution).

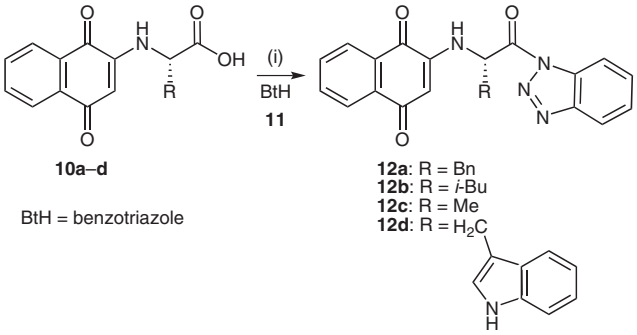
Table 1 Naphthoquinone–Amino Acid Conjugates **10a–d**

Entry	Amino acid **9**	Conjugate **10**	Yield (%)	Mp (°C)
1	L-phenylalanine (**9a**)	**10a**	72	200–203
2	L-leucine (**9b**)	**10b**	79	115–117
3	L-alanine (**9c**)	**10c**	64	137–139[a]
4	L-tryptophan (**9d**)	**10d**	58	208–211[b]

[a] Lit.[6] 139.0–142.0 °C.
[b] Lit.[20] 210.0–213.0 °C.

Naphthoquinone–amino acid conjugates **10a–d** were activated by conversion into naphthoquinone aminoacyl benzotriazole derivatives **12a–d** in 83–87% yield. The conversion of **10** into **12** was initially attempted with (i) BtH, $SOCl_2$, THF at room temperature for 2–5 hours and (ii) $BtSO_2Me$, THF, Et_3N, reflux, 8–12 hours, but complex mixtures were obtained in each case. Finally, the *N*-acyl benzotriazole derivatives were obtained in dichloromethane at room temperature in four hours using *N*,*N*-dicyclohexylcarbodiimide (DCC) as the coupling agent (Scheme 3, Table 2). The products were isolated without column chromatographic purification. The benzotriazole derivatives did not pass elemental analysis (^{1}H NMR showed minor amounts of DCU); hence, the compounds were used directly for dipeptide formation. The use of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent produced equally good results, but the high sensitivity toward moisture of the EDC hydrochloride salt led to a limited shelf-life, and therefore DCC was preferred.

The coupling of *N*-acylbenzotriazole derivatives (**12a–d**) with various natural amino acids (**9a–f**) in aqueous acetonitrile–triethylamine at 20 °C for four hours, yielded naphthoquinone dipeptides **13a–j** in good to excellent yields (Scheme 4, Table 3).



Scheme 3 *Reagents and conditions*: (i) DCC, CH_2Cl_2, r.t., 4 h.

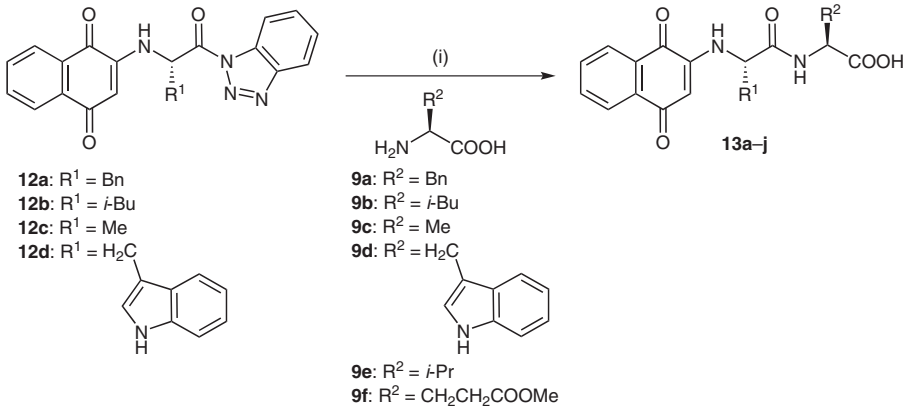
Table 2 Naphthoquinone Amino Acyl Benzotriazoles **12a–d**

Entry	Conjugate **10**	Benzotriazole **12**	Yield (%)	Mp (°C)
1	**10a**	**12a**	86	115–117
2	**10b**	**12b**	84	–[a]
3	**10c**	**12c**	87	–[a]
4	**10d**	**12d**	83	114–115

[a] Not isolated in pure form; used crude for the next coupling reaction.

All the reactions were carried at 20 °C and the products were isolated by washing the reaction mixtures with 4 M HCl to remove benzotriazole followed by precipitation of the organic layer with hexane.

In summary, we report a general method for the syntheses of naphthoquinone-dipeptides via benzotriazole methodology and have applied it to several combinations of amino acids. The previous reported method is restricted to the



Scheme 4 *Reagents and conditions*: (i) Et_3N, MeCN–H_2O, r.t., 4 h (for designation of **13a–j**, see Table 3).

synthesis of naphthoquinone-glycyl-glycine dipeptides in moderate yield. Our method allows the use of free amino acids as coupling components, does not require anhydrous conditions and, thus, is cost-effective. The advantages of using Bt-activation include: (a) excellent yields, (b) simple preparative and purification procedures, (c) short reaction times, (d) applicability to a variety of amino acids, (e) low cost, and (f) use of aqueous conditions.

Table 3 Synthesis of Naphthoquinone-dipeptides

12	Amino acid **9**	NQ–dipeptide **13**	Yield (%)	Mp (°C)
12a	L-valine (**9e**)	**13a**	81	175–177
12a	L-tryptophan (**9d**)	**13b**	81	215–217
12b	L-phenylalanine (**9a**)	**13c**	78	161–164
12b	L-tryptophan (**9d**)	**13d**	81	223–225
12b	L-alanine (**9c**)	**13e**	89	173–174
12b	L-glutamic acid methyl ester (**9f**)	**13f**	81	153–155
12c	L-tryptophan (**9d**)	**13g**	82	243–245
12d	L-leucine (**9b**)	**13h**	79	114–120
12d	L-glutamic acid methyl ester (**9f**)	**13i**	76	104–111
12d	L-phenylalanine (**9a**)	**13j**	81	121–123

Melting points were determined with a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded in $CDCl_3$, acetone-d_6 or DMSO-d_6 with TMS as internal reference for ^1H (300 MHz) and ^{13}C (75 MHz) nuclei.

Naphthoquinone–Amino Acid Conjugates 10a–d; General Procedure

To a solution of 2-naphthalene-1,4-dione (**5a**; 20 mmol) dissolved in EtOH (200 mL), L-amino acid (**9a–d**; 10 mmol), Et_3N (20 mmol) and H_2O (10 mL) were added, and the mixture was stirred at r.t. for 12 h. The resulting solution was dried under reduced pressure and the residue was subjected to column chromatography. Elution with EtOAc–hexane (2:8), removed the nonpolar impurities, and elution with 100% EtOAc, yielded naphthoquinone–amino acid triethylammonium salts, which were subsequently dissolved in CH_2Cl_2 (50 mL), and washed with 4 M HCl (3 × 30 mL) and then brine (2 × 30 mL). The organic layers were dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was dried overnight under vacuum to yield the corresponding naphthoquinone–amino acid conjugates **10a–d**.

(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-phenylpropanoic Acid (10a)

Yield: 2.31 g (72%); black solid; mp 200.0–203.0 °C.

^1H NMR (300 MHz, DMSO-d_6): δ = 3.22 (t, *J* = 7.5 Hz, 2 H), 4.48–4.54 (m, 1 H), 5.74 (s, 1 H), 7.01–7.26 (m, 6 H), 7.74 (t, *J* = 7.5 Hz, 1 H), 7.84 (t, *J* = 7.5 Hz, 1 H), 7.91 (d, *J* = 7.5 Hz, 1 H), 7.96 (d, *J* = 7.5 Hz, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 35.9, 55.7, 101.2, 125.6, 126.2, 126.8, 128.4, 129.4, 130.3, 132.6, 132.9, 135.2, 137.0, 147.6, 172.0, 181.4, 182.0.

(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-4-methylpentanoic Acid (10b)

Yield: 2.27 g (79%); black solid; mp 115.0–117.0 °C.

^1H NMR (300 MHz, DMSO-d_6): δ = 0.87 (d, *J* = 6.0 Hz, 3 H), 0.92 (d, *J* = 6.3 Hz, 3 H), 1.65–1.71 (m, 2 H), 1.88–1.96 (m, *J* = 8.4 Hz, 1 H), 4.07–4.11 (m, 1 H), 5.68 (s, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.74 (td, *J* = 1.5, 7.5 Hz, 1 H), 7.83 (td, *J* = 1.5, 7.5 Hz, 1 H), 7.94 (dd, *J* = 1.2, 7.5 Hz, 1 H), 8.00 (dd, *J* = 1.2, 7.5 Hz, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.6, 22.7, 24.6, 53.4, 100.7, 125.4, 126.0, 130.3, 132.5, 132.8, 134.9, 148.1, 172.9, 181.3, 181.7.

HRMS: *m/z* $[M + H]^+$ calcd for $C_{16}H_{18}NO_4$: 288.1320; found: 288.1233.

(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)propanoic Acid (10c)

Yield: 1.57 g (64%); red solid; mp 137.0–139.0 °C (Lit.[6] 139.0–142.0 °C).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.31 (d, *J* = 2.7 Hz, 3 H), 3.60–3.80 (m, 1 H), 5.58 (s, 1 H), 7.43 (d, *J* = 6.0 Hz, 1 H), 7.71 (t, *J* = 7.5 Hz, 1 H), 7.82 (t, *J* = 7.2 Hz, 1 H), 7.92–7.99 (m, 2 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 17.1, 51.5, 99.1, 125.3, 125.8, 130.2, 132.0, 133.3, 134.8, 146.5, 173.6, 181.0, 181.6.

(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-(1*H*-indol-3-yl)propanoic Acid (10d)
Yield: 2.09 g (58%); black solid; mp 208.0–211.0 °C (Lit.[19] 210.0–213.0 °C).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.36–3.38 (m, 2 H), 4.45–4.52 (m, 1 H), 5.74 (s, 1 H), 6.91–6.97 (m, 2 H), 7.05 (t, *J* = 7.8 Hz, 1 H), 7.18 (d, *J* = 2.4 Hz, 1 H), 7.32 (dd, *J* = 0.6, 8.1 Hz, 1 H), 7.52 (d, *J* = 8.1 Hz, 1 H), 7.74 (t, *J* = 7.5 Hz, 1 H), 7.83 (t, *J* = 7.5 Hz, 1 H), 7.92–8.00 (m, 2 H), 10.90 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.1, 55.2, 100.9, 108.8, 111.4, 118.1, 118.4, 120.9, 124.0, 125.3, 125.9, 127.2, 130.0, 132.3, 132.7, 134.9, 136.0, 147.2, 172.1, 181.1, 181.7.

Naphthoquinone–Amino Acyl Benzotriazole Derivatives 12a–d; General Procedure
To a solution of naphthoquinone–amino acid conjugate (**10a–d**; 5 mmol) in anhydrous CH_2Cl_2 (30 mL), benzotriazole (**11**; 5 mmol, 595 mg) and DCC (5 mmol, 1.03 g) were added. The reaction mixtures were stirred at r.t. for 4 h and then filtered through Celite at least twice. The organic layers were concentrated in vacuo and the residues were recrystallized from EtOAc–hexane to yield naphthoquinone-aminoacyl benzotriazole derivatives **12a–d**.

(*S*)-2-{1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-ylamino}naphthalene-1,4-dione (12a)
Yield: 1.82 g (86%); black solid; mp 115.0–117.0 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.37 (dd, *J* = 7.5, 13.8 Hz, 1 H), 3.60 (dd, *J* = 4.8, 13.8 Hz, 1 H), 5.76 (s, 1 H), 5.56–5.82 (m, 1 H), 6.55 (d, *J* = 7.8 Hz, 1 H), 7.17–7.33 (m, 5 H), 7.54–7.72 (m, 4 H), 8.04 (t, *J* = 6.0 Hz, 2 H), 8.20 (t, *J* = 7.5 Hz, 2 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.9, 56.6, 103.0, 114.4, 120.8, 126.4, 126.6, 127.1, 127.9, 129.1, 129.3, 130.5, 131.0, 131.3, 132.5, 133.2, 134.8, 134.9, 146.3, 146.6, 169.6, 181.3, 183.3.

(*S*)-2-{1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-3-(1*H*-indol-3-yl)-1-oxopropan-2-ylamino}naphthalene-1,4-dione (12d)
Yield: 1.92 g (83%); yellow solid; mp 114.0–115.0 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.60 (dd, *J* = 7.6, 15.0 Hz, 1 H), 3.80 (dd, *J* = 4.8, 14.7 Hz, 1 H), 5.72 (s, 1 H), 5.92 (q, *J* = 4.8 Hz, 1 H), 6.61 (d, *J* = 8.1 Hz, 1 H), 7.00 (t, *J* = 7.5 Hz, 1 H), 7.10–7.17 (m, 2 H), 7.29 (d, *J* = 8.4 Hz, 1 H), 7.45 (d, *J* = 7.8 Hz, 1 H), 7.54–7.72 (m, 5 H), 8.02 (d, *J* = 7.8 Hz, 1 H), 8.18 (t, *J* = 6.9 Hz, 2 H), 8.26 (br s, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.3, 56.1, 103.0, 111.7, 114.6, 118.5, 120.3, 120.8, 122.9, 123.7, 126.5, 126.7, 127.1, 131.4, 132.6, 135.0, 147.0, 170.1.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{27}H_{20}N_5O_3$: 462.1561; found: 462.1556.

Naphthoquinone–Dipeptides 13a–j; General Procedure
A solution of L-amino acid (1 mmol; **9a–f**) and Et_3N (1.2 mmol) in H_2O (4 mL) was added to a solution of *N*-acyl benzotriazole derivative (1 mmol; **12a–d**) in MeCN (50 mL). The reaction was stirred at r.t. for 3–4 h and then quenched with 4 M HCl (2 mL). The reaction mixture was concentrated, diluted with EtOAc (100 mL), and washed with 4 M HCl (3 × 30 mL), and brine (2 × 30 mL). The organic layer was concentrated and cold hexane (30 mL) was added to the resulting solution. The precipitated solid was filtered and dried under vacuum to yield naphthoquinone–dipeptides **13a–j**.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-phenylpropanamido]-3-methylbutanoic Acid (13a)
Yield: 0.34 g (81%); red solid; mp 175.0–177.0 °C.

^{1}H NMR (300 MHz, acetone-d_6): δ = 0.97 (t, *J* = 5.4 Hz, 6 H), 2.16–2.27 (m, 1 H), 3.23 (dd, *J* = 7.8, 13.8 Hz, 1 H), 3.36 (dd, *J* = 5.1, 13.8 Hz, 1 H), 4.47–4.60 (m, 2 H), 5.72 (s, 1 H), 6.68 (d, *J* = 7.2 Hz, 1 H), 7.16–7.34 (m, 5 H), 7.65–7.81 (m, 2 H), 7.83 (t, *J* = 13.9 Hz, 1 H), 8.02 (t, *J* = 7.5 Hz, 2 H).

^{13}C NMR (75 MHz, acetone-d_6): δ = 18.2, 19.5, 31.6, 38.6, 57.77, 57.84, 58.1, 102.6, 126.5, 126.6, 126.8, 127.7, 129.3, 129.5, 130.3, 131.5, 133.1, 134.2, 135.5, 137.7, 147.9, 170.9, 172.8, 182.2, 182.7.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{24}H_{25}N_2O_5$: 421.1758; found: 421.1778.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-phenylpropanamido]-3-(1*H*-indol-3-yl)propanoic Acid (13b)
Yield: 0.41 g (81%); red solid; mp 215.0–217.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.06–3.14 (m, 3 H), 3.23 (dd, *J* = 5.1, 5.4 Hz, 1 H), 4.31–4.38 (m, 1 H), 4.52–4.59 (m, 1 H), 5.57 (s, 1 H), 6.93 (t, *J* = 7.2 Hz, 1 H), 7.00–7.04 (m, 1 H), 7.14–7.24 (m, 5 H), 7.32 (d, *J* = 7.8 Hz, 2 H), 7.53 (d, *J* = 7.8 Hz, 2 H), 7.73 (t, *J* = 7.5 Hz, 1 H), 7.82 (t, *J* = 7.5 Hz, 1 H), 7.91 (d, *J* = 7.2 Hz, 1 H), 7.97 (d, *J* = 7.5 Hz, 1 H), 8.65 (d, *J* = 8.1 Hz, 1 H), 10.87 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.1, 37.1, 53.0, 56.6, 100.9, 109.5, 111.3, 116.4, 118.1, 118.3, 120.8, 123.6, 124.5, 125.3, 125.7, 125.9, 126.0, 126.4, 127.1, 128.1, 128.5, 129.1, 130.0, 132.3, 132.6, 134.8, 136.0, 136.9, 147.2, 169.6, 172.8, 180.9, 181.6.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{26}N_3O_5$: 508.1867; found: 508.1886.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-4-methylpentanamido]-3-phenylpropanoic Acid (13c)
Yield: 0.34 g (78%); red solid; mp 161.0–164.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.83 (d, *J* = 3.0 Hz, 3 H), 0.90 (d, *J* = 3.0 Hz, 3 H), 1.47–1.62 (m, 2 H), 1.70–1.78 (m, 1 H), 2.89 (dd, *J* = 6.3, 13.8 Hz, 1 H), 3.08 (dd, *J* = 4.5, 13.8 Hz, 1 H), 3.98–4.05 (m, 1 H), 4.44–4.50 (m, 1 H), 5.74 (s, 1 H), 6.99 (d, *J* = 8.4 Hz, 1 H), 7.06–7.11 (m, 1 H), 7.15–7.20 (m, 4 H), 7.75 (t, *J* = 7.5 Hz, 1 H), 7.85 (t, *J* = 7.5 Hz, 1 H), 7.95–8.02 (m, 2 H), 8.51 (d, *J* = 8.1 Hz, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.7, 22.4, 24.1, 36.4, 53.1, 54.1, 100.7, 125.1, 125.7, 126.0, 127.8, 128.9, 130.0, 132.2, 132.5, 134.7, 137.0, 147.2, 170.4, 172.2, 180.9, 181.5.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{25}H_{27}N_2O_5$: 435.1914; found: 435.1934.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-4-methylpentanamido]-3-(1*H*-indol-3-yl)propanoic Acid (13d)
Yield: 0.38 g (81%); red solid; mp 223–225.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.82 (d, *J* = 6.3 Hz, 3 H), 0.89 (d, *J* = 6.0 Hz, 3 H), 1.52–1.61 (m, 2 H), 1.64–1.75 (m, 1 H), 3.06 (dd, *J* = 5.4, 14.4 Hz, 1 H), 3.19 (dd, *J* = 5.4, 14.4 Hz, 1 H), 4.01–4.08 (m, 1 H), 4.51–4.54 (m, 1 H), 5.73 (s, 1 H), 6.90–7.03 (m, 3 H), 7.14 (d, *J* = 2.1 Hz, 1 H), 7.29 (d, *J* = 7.5 Hz, 1 H), 7.51 (td, *J* = 1.5, 7.5 Hz, 1 H), 7.74 (td, *J* = 1.5, 7.5 Hz, 1 H), 7.84 (td, *J* = 1.2, 7.5 Hz, 1 H), 7.95 (dd, *J* = 1.2, 8.1 Hz, 1 H), 8.00 (dd, *J* = 1.2, 7.8 Hz, 1 H), 8.47 (d, *J* = 8.1 Hz, 1 H), 10.81 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.0, 22.7, 24.3, 53.0, 54.4, 100.8, 109.6, 111.4, 118.1, 118.3, 120.8, 123.6, 125.4, 126.0, 127.2, 130.3, 132.4, 132.8, 134.9, 136.1, 147.6, 170.7, 172.9, 181.2, 181.8.

Anal. Calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.58; H, 5.51; N, 8.45.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-4-methylpentanamido]propanoic Acid (13e)
Yield: 0.32 g (89%); orange solid; mp 173.0–174.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.85 (d, *J* = 6.3 Hz, 3 H), 0.92 (d, *J* = 6.3 Hz, 3 H), 1.23 (d, *J* = 7.2 Hz, 3 H), 1.55–1.69 (m, 2 H), 1.69–1.80 (m, 1 H), 4.05–4.12 (m, 2 H), 5.73 (s, 1 H), 7.12 (d, *J* = 8.4 Hz, 1 H), 7.73 (t, *J* = 7.5 Hz, 1 H), 7.83 (t, *J* = 7.5 Hz, 1 H), 7.93 (d, *J* = 7.5 Hz, 1 H), 7.99 (d, *J* = 7.5 Hz, 1 H), 8.19 (d, *J* = 6.9 Hz, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 18.3, 21.9, 22.8, 24.4, 48.8, 54.5, 100.7, 125.4, 126.0, 130.3, 132.4, 132.8, 134.9, 147.8, 169.9, 181.2, 181.8.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{19}H_{23}N_2O_5$: 359.1601; found: 359.1596.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-4-methylpentanamido]-5-methoxy-5-oxopentanoic Acid (13f)
Yield: 0.35 g (81%); red solid; mp 153.0–154.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.86 (d, *J* = 6.3 Hz, 3 H), 0.92 (d, *J* = 6.0 Hz, 3 H), 1.22–1.26 (m, 1 H), 1.58–1.71 (m, 2 H), 1.76–1.85 (m, 2 H), 2.00–2.03 (m, 1 H), 2.29–2.35 (m, 1 H), 3.51 (s, 3 H), 4.00–4.16 (m, 2 H), 5.75 (s, 1 H), 7.12 (d, *J* = 8.7 Hz, 1 H), 7.73 (t, *J* = 7.5 Hz, 1 H), 7.83 (t, *J* = 7.5 Hz, 1 H), 7.93 (d, *J* = 7.5 Hz, 1 H), 7.99 (d, *J* = 7.2 Hz, 1 H), 8.22 (d, *J* = 7.5 Hz, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.9, 21.9, 22.1, 22.8, 24.4, 26.9, 29.7, 31.0, 51.2, 52.0, 54.5, 100.9, 125.4, 126.0, 130.3, 132.4, 132.8, 134.9, 147.7, 170.4, 172.9, 181.3, 181.7.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{22}H_{27}N_2O_7$: 431.1813; found: 431.1816.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)propanamido]-3-(1*H*-indol-3-yl)propanoic Acid (13g)
Yield: 0.35 g (82%); red solid; mp 243.0–245.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.36 (d, *J* = 6.6 Hz, 3 H), 3.08 (dd, *J* = 5.1, 14.7 Hz, 1 H), 3.22 (dd, *J* = 5.1, 14.7 Hz, 1 H), 4.12 (t, *J* = 7.2 Hz, 1 H), 4.50–4.57 (m, 1 H), 5.60 (s, 1 H), 6.93–7.08 (m, 3 H), 7.16 (d, *J* = 2.1 Hz, 1 H), 7.31 (d, *J* = 7.8 Hz, 1 H), 7.52 (d, *J* = 7.8 Hz, 1 H), 7.75 (td, *J* = 1.2, 7.5 Hz, 1 H), 7.85 (td, *J* = 1.2, 7.5 Hz, 1 H), 7.95 (d, *J* = 6.6 Hz, 1 H), 8.00 (d, *J* = 6.9 Hz, 1 H), 8.50 (d, *J* = 8.1 Hz, 1 H), 10.85 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 17.6, 27.0, 50.8, 52.9, 100.7, 109.5, 111.3, 118.0, 118.3, 120.8, 123.5, 125.3, 125.9, 127.1, 130.2, 132.3, 132.8, 134.8, 136.0, 147.0, 171.0, 172.8, 181.1, 181.6.

Anal. Calcd for $C_{24}H_{21}N_3O_5$: C, 66.81; H, 4.91; N, 9.74. Found: C, 66.57; H, 4.79; N, 9.50.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-(1*H*-indol-3-yl)propanamido]-4-methylpentanoic Acid (13h)
Yield: 0.37 g (79%); orange solid; mp 114.0–120.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.81 (d, *J* = 6.0 Hz, 3 H), 0.91 (d, *J* = 6.3 Hz, 3 H), 1.22–1.25 (m, 1 H), 1.54–1.77 (m, 2 H), 3.27–3.30 (m, 2 H), 4.28–4.38 (m, 2 H), 5.59 (s, 1 H), 6.90–6.99 (m, 2 H), 7.05 (t, *J* = 7.8 Hz, 1 H), 7.27 (s, 1 H), 7.32 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 7.8 Hz, 1 H), 7.71 (t, *J* = 7.2 Hz, 1 H), 7.80 (t, *J* = 7.5 Hz, 1 H), 7.89 (d, *J* = 7.5 Hz, 1 H), 7.94 (d, *J* = 7.5 Hz, 1 H), 8.61 (d, *J* = 7.8 Hz, 1 H), 10.89 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.0, 22.8, 24.3, 27.4, 33.3, 38.6, 50.2, 56.1, 100.9, 109.0, 111.3, 118.2, 118.3, 120.9, 124.2, 125.3, 125.8, 127.2, 130.1, 132.3, 132.7, 134.8, 136.1, 147.3, 170.2, 173.7, 181.0, 181.5.

Anal. Calcd for $C_{27}H_{27}N_3O_5$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.20; H, 5.90; N, 8.47.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-(1*H*-indol-3-yl)propanamido]-5-methoxy-5-oxopentanoic Acid (13i)
Yield: 0.38 g (76%); yellow solid; mp 104.0–111.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.85–1.92 (m, 1 H), 1.98–2.15 (m, 1 H), 2.37 (t, *J* = 7.2 Hz, 2 H), 3.29–3.38 (m, 2 H), 3.56 (s, 3 H), 4.26–4.40 (m, 2 H), 5.61 (s, 1 H), 6.96 (t, *J* = 7.2 Hz, 2 H), 7.02 (t, *J* = 7.2 Hz, 1 H), 7.25 (s, 1 H), 7.31 (d, *J* = 8.1 Hz, 1 H), 7.62 (d, *J* = 7.8 Hz, 1 H), 7.72 (t, *J* = 7.2 Hz, 1 H), 7.81 (t, *J* = 7.2 Hz, 1 H), 7.89 (d, *J* = 7.5 Hz, 1 H), 7.95 (d, *J* = 7.5 Hz, 1 H), 8.59 (d, *J* = 7.8 Hz, 1 H), 10.88 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.2, 27.3, 29.6, 51.1, 51.2, 56.1, 100.8, 109.0, 111.3, 118.1, 118.3, 120.9, 124.1, 125.3, 125.8, 127.2, 130.1, 132.3, 132.7, 134.8, 136.1, 147.3, 170.3, 172.5, 181.3, 181.5.

Anal. Calcd for $C_{27}H_{25}N_3O_7$: C, 64.41; H, 5.00; N, 8.35. Found: C, 64.09; H, 5.05; N, 7.98.

(*S*)-2-[(*S*)-2-(1,4-Dioxo-1,4-dihydronaphthalen-2-ylamino)-3-(1*H*-indol-3-yl)propanamido]-3-phenylpropanoic Acid (13j)
Yield: 0.41 g (81%); yellow solid; mp 121.0–123.0 °C.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.94–3.00 (m, 1 H), 3.05–3.18 (m, 1 H), 3.20–3.28 (m, 2 H), 4.20–4.35 (m, 1 H), 4.51–4.55 (m, 1 H), 5.61 (s, 1 H), 6.90–7.01 (m, 2 H), 7.07 (t, *J* = 6.9 Hz, 1 H), 7.12–7.25 (m, 6 H), 7.33 (d, *J* = 7.8 Hz, 1 H), 7.64 (d, *J* = 7.5 Hz, 1 H), 7.71 (d, *J* = 6.3 Hz, 1 H), 7.80 (t, *J* = 7.5 Hz, 1 H), 7.90–7.94 (m, 2 H), 8.70 (d, *J* = 7.2 Hz, 1 H), 10.88 (s, 1 H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.9, 37.3, 54.0, 56.7, 101.4, 109.7, 111.9, 118.7, 118.8, 121.5, 124.6, 125.8, 126.4, 126.9, 127.7, 128.6, 129.6, 130.6, 132.8, 133.2, 135.4, 136.6, 137.8, 147.8, 170.7, 173.0, 181.5, 182.1.

Anal. Calcd for $C_{30}H_{25}N_3O_5$: C, 70.99; H, 4.96; N, 8.28. Found: C, 71.26; H, 5.29; N, 7.85.

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