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A Combinatorial Approach to the Synthesis of Cystine Based Organogelators

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Abstract: A solid-phase approach was used to prepare 20 cystine amide derivatives with disulfide bond formation resulting from an intra-site reaction between neighbouring cysteine residues. Library members were screened as potential organogelators in a range of solvent mixtures and resulted in the identification of a potent gelator able to rigidify water/DMSO mixtures at concentrations as low as 1.3 mM. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: solid-phase synthesis; cystine; organogelators; intra-site reactions; combinatorial chemistry; disulfide bridges; cysteine

INTRODUCTION

Most of the gels known originate by mixing solvents with a range of polymeric compounds such as gelatine, pectin and agarose. Modern organic chemists routinely come across inorganic gels prepared by swelling silica in organic solvents or those obtained from swollen organic polymers such as polystyrene-divinylbenzene which now have widespread use in solid phase organic synthesis. Recently, however, there has been a growing interest in gelation induced by relatively low molecular weight (MW $\leq$ 3000) organic molecules ('gelators') that are capable of gelating, thermoreversibly, organic solvents at low concentrations (less than 5% w/w) [1–4]. These low molecular weight organogelators are receiving attention because of the challenges related to the description of their self-assembling properties and because of their potential in many applications of chemistry, including food processing, cosmetics, drug delivery and biocompatible materials [1–4]. The discovery of new classes of gelating agents still remains serendipitous due to the lack of understanding both of the nature of the nucleation processes and the factors and interactions responsible for gel stabilization. Combinatorial chemistry seems to highly amenable to use in this field due to the possibility of synthesizing large arrays of compounds and of developing high-throughput screens for gelation in order to discover new compounds which gelate mixtures of solvents at minimum concentration but with maximum gel strength. The preparation of libraries of compounds is a tool which has been used to study the structure–gelation ability relationships of classes of potential organogelators in order to obtain insights on the functional groups (i.e. interactions) which promote and stabilize gel formation [5,6]. In the present study, a parallel solid phase approach represented a flexible and highly advantageous tool in order to create libraries of compounds that could be screened as organogelators following cleavage from the resin. For this purpose, it was crucial that a core structure could be identified and diversified at one or more points on the solid phase. Acyl cystine

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derivatives were ideal candidates for this study, as the exceptional ability of cystine derivatives to gelate water mixtures has been well documented [7–10] and their structures include sites that can be easily diversified such as the *C* and *N* terminus.

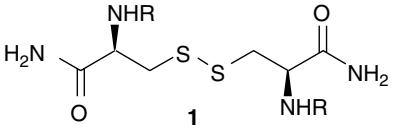
RESULTS AND DISCUSSION

The synthetic procedures used to access the library (Table 1) are outlined in Scheme 1. In order to test the efficiency of this approach, two known cystine organogelators [10] **1a** and **1b** were initially prepared and screened for their organogelating ability.

Fmoc-Cys(Trt)-OH was thus coupled to Rink amide PS-resin (prepared by coupling the Knorr linker to aminomethyl resin (1% DVB, 1.65 mmol/g)). After deprotection of the Fmoc group, the resin was capped with either *p*-methoxy benzoic acid **3a** or 2-naphthoic acid **3b**. Disulfide bond formation was carried out on resin with I_2 *via* an intra-resin site–site reaction (for reviews [11–15]) using well documented chemistry of S-Trt-cysteine containing peptides [16,17], iodine not only cleaving the S-Trt protecting group but also allowing homodimerization of the precursor resin bound thiols.

The resin bound cystine derivatives were cleaved from the resin with a TFA cocktail and obtained in good yield and purity (**1a**, yield: 85%, purity: 91% (RP-HPLC (ELS detector); **1b**, yield: 55%, purity: 80% (RP-HPLC (ELS detector)). In order to ensure that dimerization was a result of site–site reaction and did not occur during cleavage and work-up, resin **2a**, capped with *p*-methoxy benzoic acid, was exposed to the TFA cocktail before and after the iodine treatment. RP-LC-MS analyses of the crude

Table 1 Library of *N*,*N*′-Diacylcystine based Derivatives



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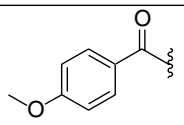
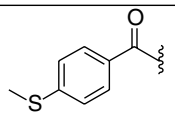
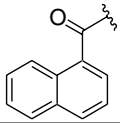
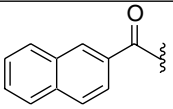
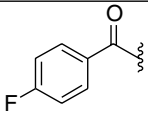
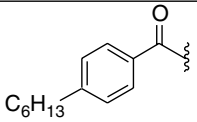
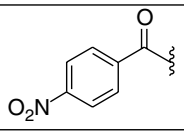
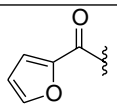
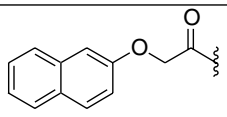
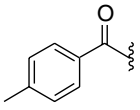
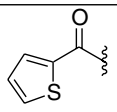
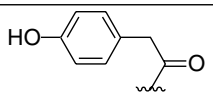
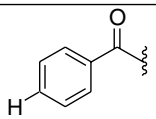
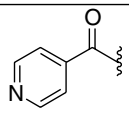
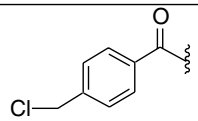
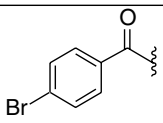
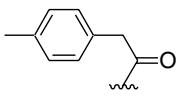
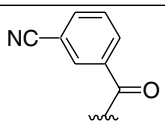
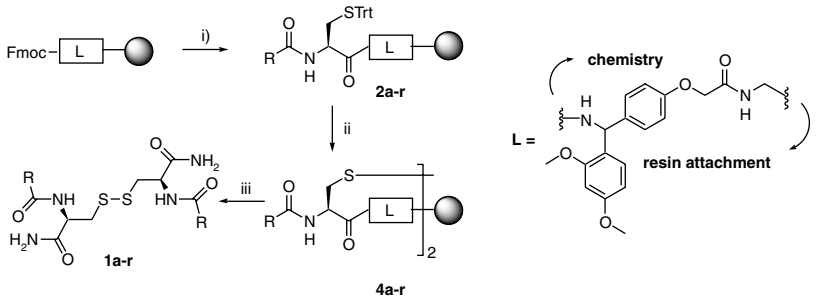
a		**g**		**m**	
b		**h**		**n**	
c		**i**		**o**	
d		**j**		**p**	
e		**k**		**q**	
f		**l**		**r**	



(i) (a) 20% piperidine in DMF; (b) Fmoc-Cys(Trt)-OH/DIC/HOBt (2/2.2/2 equiv) in CH_2Cl_2/DMF; (c) 20% piperidine in DMF; (d) RCOOH (**3a-t**)/DIC/HOBt (2/2.2/2 equiv) in CH_2Cl_2/DMF; (ii) I_2 (10 equiv) in DMF; (iii) TFA/TIS/CH_2Cl_2 (95/2/3).

Scheme 1 Solid-phase synthetic procedure to access cystine derivatives.

material cleaved from resin, indicated the presence of the dianisoyl cystine species **1a** only in the sample treated with iodine (Figure 1).

1a gelated water/5% DMSO solution at a concentration of 2.6 mM (0.13% w/w), giving a soft gel at 2 mM, in good agreement with previous observations†.

Cystine **1b** was found to rigidify aqueous solutions at 0.25 mM, (ca. 0.01% w/w) in less than 30 s [10], representing one of the lowest values obtained for low molecular weight organogelators. Crude **1b** was screened in water/25% DMSO mixtures (according to the method reported in the literature) and was found to gelate the water rich mixture at the remarkably low concentration of 1.8 mM while giving a soft gel/liquid when the volume was doubled.

These results encouraged the preparation of a library of cystine amides as potential organogelators in order to optimize structures which allowed minimization of the concentration required for gelation.

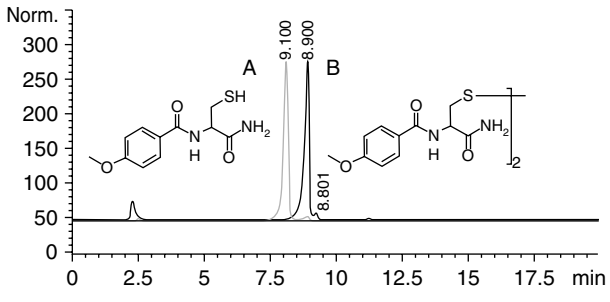


Figure 1 RP-HPLC (ELS-detector) traces of the cleaved product: (a) before iodine treatment; (b) after iodine treatment.

† In a previous study, [10] compound **1a** was able to gelate this polar system at the remarkable concentration of 2 mM.

Most members **1c–r** were efficiently prepared *via* solid-phase synthesis (Scheme 1) and were identified and characterized by RP-LC-MS (yields and purities of the crude members are given in Table 2).

The cystine derivatives were screened in a selection of solvents with different properties and/or hydrogen bond donor/acceptor abilities, such as water, ethanol, TEOS (tetraethyl orthosilicate, a solvent used in sol-gel polycondensation processes [4],) acetonitrile, toluene, ethyl acetate, chloroform and acetic acid.

In a typical gelation screening experiment, the potential organogelator was placed into a glass vial, a known volume of solvent added (starting concentration: 2.6% w/w) and the sample sonicated or heated for 10 min. The samples were allowed to cool and subsequently inverted to observe (visual inspection) their flow properties. The sample was then diluted until gel formation ceased in that particular solvent (Table 3). Images were taken of each set of screening experiments for all members at different concentrations (two library members **1d** and **1p** gave complex mixtures and thus screening data for these compounds is excluded from the table.

The common drawback of this screening method was the insolubility of many of the mixtures in the chosen solvent (precipitation and gelation are often competing events: one of the theories elaborated to explain the gelation phenomenon refers to the thickening of an organic solvent as a result of *incomplete crystallization* of solute [10].) In most cases, gelation concentrations were in the range 2.6–1.3% w/w (Table 3). It was also found that heteroaromatic derivatization of cystine gave very poor gelators (Table 3, substrates **1i–k**) with only very soft gels forming together with precipitates.

Table 2 Yields and Purities of Cystine Derivatives **1a–r** after Cleavage from Resin

1	Yield (%)[a]	Purity (%)[b]	**1**	Yield (%)[a]	Purity (%)[b]	**1**	Yield (%)[a]	Purity (%)[b]
a	85	91	**g**	66	100	**m**	21	95
b	90	55	**h**	57	100	**n**	44	100
c	50	100	**i**	73	/[d]	**o**	22	90
d	61	/[c]	**j**	30	100	**p**	52	/[c]
e	70	100	**k**	38	91	**q**	57	100
f	44	100	**l**	50	64[e]	**r**	41	83

[a] Based on starting loading of aminomethyl resin (1.65 mmol/g), assuming that the recovered solid was 100% the expected cystine derivative.
[b] Based on LC-MS analysis, UV detector ($\lambda = 254$ nm).
[c] Very complex mixture.
[d] Two components were obtained which were not separable on the LC and were not resolved in the TIC profile.
[e] Based on LC-MS analysis, UV detector ($\lambda = 220$ nm).

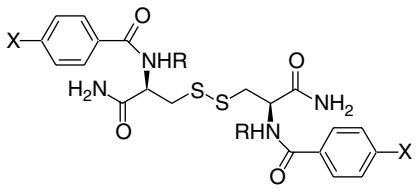
Table 3 Gelation Test Data

1	Solvent								c (%, w/w)
	H_2O	EtOH	TEOS	MeCN	Toluene	EtOAc	$CHCl_3$	AcO	
c	P	G/P	P	SG	SG	SG	G	G/P	2.6
e	M	**G**	P	**G**	M	M	M	**G**	2.6
f	SG	G	M	M	**G**	**G**	S	M	1.3
g	M	**G**	P	M	M	M	M	**G**	1.3
h	SG	**G**[a]	M	**G**	SG	**G**	**G**	S	1.3
j	M	M	M	M	SG	P	M	P	2.6
k	S	P	P	M	P	M	SG	S	2.6
l[b]	P	**G**	P	SG	M	SG	**G**	S	2.6
m	P	SG	P	M	M	M	SG	SG/S	2.6
n	P	SG	P	SG	M	**G**	M	S	1.3
o	M	SG/P	SG/P	G/P	M	G/P	**G**	S	2.6
q	M	**G**	M	SG/P	SG	SG	S	S	1.3
r	P	**G**	P	**G**	M	**G**	M	M	2.6

G: opaque gel; P: precipitate; SG: opaque soft gel; M: solvent, precipitate and 'jelly' aggregates; S: solution;
[a] 0.67% w/w mixtures.
[b] Only 64% pure by HPLC. **1d**, **i**, **p** excluded as complex mixtures.

With the *p*-fluorobenzoyl derivative **1h**, gelation occurred in pure ethanol at a low concentration (0.89% w/w, 18 mM).

The *p*-bromo analogue **1f** also showed good gelation behaviour in ethanol, toluene and ethyl acetate at a concentration of 1.3% w/w (22 mM). Intrigued by the behaviour of the *p*-halogen derivatives, four *p*-halogen dibenzoyl cystine derivatives were synthesized (Figure 2) by the solid-phase approach outlined earlier and screening carried out in water/DMSO.



X = F(**1h**), Cl(**1s**), Br(**1f**), I(**1t**)

Figure 2 *p*-halogen benzoyl derivatives.

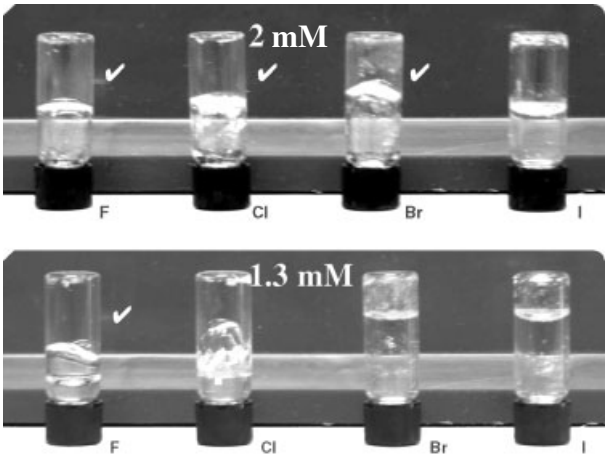


Figure 3 Screening of *p*-halogen derivatives in water/10%DMSO.

The four halogen derivatives **1f**, **h**, **s**, **t** were screened in water/10% DMSO mixture (Figure 3) (a) and (b)). At a concentration of 0.097% w/w (2 mM), the fluoro derivative **1h** gave a thick, clear and transparent gel which softened upon lowering the concentration to 0.063% w/w (1.3 mM). **1s** gave a good gel at 0.10% w/w (2 mM) while it turned into a less viscous one at a lower concentration. The bromo and iodo analogues gave poorer results: compound **1t** gave clear solutions at both of the concentrations screened.

CONCLUSIONS

In conclusion, an efficient and rapid solid phase route to cystine derivatives has been developed and applied to the synthesis of a library of one of the most potent classes of organogelators so far discovered. The crude peptides were successfully screened and a subclass, the *p*-halogen benzoyl derivatives, showed outstanding gelating properties. In particular, a new super gelator has been identified, the *p*-fluoro analogue, which was able to gelatinize DMSO/water mixtures at the remarkably low concentration of 1.3 mM, one of the lowest values described in the literature for low molecular weight organogelators. The potential of the solid phase route is immense in terms of diversification of the core cystine structure and the immense opportunities for diversification and the possibility of using mixtures and capping groups in a deconvolution type strategy have many attractions making this strategy ideal for combinatorial and high throughput screening applications.

EXPERIMENTAL SECTION

General

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC300 (300 and 75 MHz, respectively) or on a Bruker DPX400 (400 and 100 MHz, respectively) at 298K. All chemical shifts are reported in units of δ (ppm) using the residual protonated solvent as the internal standard or ^{13}C natural abundance of the deuterated solvent. Analytical RP-HPLC were carried out on a Hewlett Packard HP1100 Chemstation eluting with (A) 0.1% TFA/H_2O and (B) 0.04% TFA/MeCN. *Method (1)* Column C_{18} ODS, 150 mm × 3 mm i.d., flow rate: 0.5 ml/min. Gradient: 0% (B) to 100% (B) over 20 min. Detection by UV (220 and 254 nm) and ELS detectors. LC-MS were carried out on an Agilent LCMSD 1100 equipped with an Agilent C_8 column, 150 mm × 4.6 mm i.d., flow rate: 0.5 ml/min eluting with (A) H_2O and (B) MeCN. *Method (2)* Gradient: 10% (B) to 90% (B) over 10 min. *Method (3)* Flow rate: 1 ml/min. Gradient: 5% (B) to 95% (B) over 10 min. ES mass spectra were recorded by using a VG Platform Quadrupole Electrospray Ionisation mass spectrometer, measuring monoisotopic masses. HRMS were run on a Bruker APEX3. Infrared spectra were recorded on a BIO-RAD FTS 135 spectrometer with a Golden gate ATR with neat compounds. UV-VIS spectra were recorded using a HP 8452 A diode array spectrophotometer. Thin layer chromatography aluminum backed silica plates (0.25 mm layer of silica gel 60 with the florescent indicator Alugram SIL G/UV_{254}) were used.

Materials

Aminomethyl polystyrene resin (1.65 mmol/g) and the Knorr linker were purchased from Novabiochem (1% DVB, 200–400 mesh). Fmoc-Cys(Trt)-OH was of the L configuration and purchased from Advanced Chemtech.

Methods

General resin procedures. The quantitative ninhydrin test [18] and the quantitative Fmoc test [19,20] were used.

General methods for SPPS (19–21). 10 ml of solvent per gram of resin was used for the following procedures.

Method A: N-terminal Fmoc removal. Fmoc removal was performed using 20% piperidine in DMF with

two sequential treatments of 20 min. The resin was then filtered and washed with DMF (×3), CH_2Cl_2 (×3), MeOH (×3), Et_2O (×2) and dried under vacuum for 30 min.

Method B: Solid phase peptide coupling conditions. The resin was swollen in a minimum amount of CH_2Cl_2. *N*-Fmoc-amino acid (2 eq) and HOBt (2 eq) were dissolved in CH_2Cl_2 with a few drops of DMF and stirred for 10 min. DIC (2.2 eq) was added and the mixture stirred for 10 min before addition to the resin. The resin was agitated for 2 h to effect coupling. The resin was washed with DMF (×3), CH_2Cl_2 (×3), MeOH (×3), Et_2O (×2) and dried under vacuum for 30 min.

Method C: Intra-resin disulfide bond formation. ArCONH-Cys(Trt)-Rink amide resin (500 mg) was shaken with a solution of iodine (10 eq) in DMF for 1.5 h. The resin was then washed with DMF (10 ml × 5), CH_2Cl_2 (10 ml × 5), MeOH (10 ml × 5) and Et_2O (10 ml × 3).

Method D: Cleavage of peptides from resin. The resin was swollen in a minimum CH_2Cl_2. TFA/TIS/CH_2Cl_2 (95/2/3) was added and the resin agitated for 2 h. The TFA solution was removed, concentrated to ca. 1 ml and added to cold Et_2O in a centrifuge tube. The resulting precipitate was collected by centrifugation and washed with Et_2O (×4).

H-RINK-PS. The Fmoc-Knorr-polystyrene resin (loading 0.89 mmol/g, by quantitative Fmoc test) was deprotected according to Method A. Qualitative ninhydrin test positive.

Fmoc-Cys(Trt)-Rink-PS. Rink amide AM resin (9.34 g, 10.3 mmol, 1 eq) was coupled to Fmoc-Cys(Trt)-OH (12.1 g, 20.6 mmol, 2 eq) according to Method B. Qualitative ninhydrin test negative. Quantitative Fmoc test gave a loading of 0.61 mmol/g (theoretical loading: 0.69 mmol/g).

H-Cys(Trt)-Rink-PS. The Fmoc-Cys(Trt)-Rink-PS resin (0.50 g, 0.34 mmol) was deprotected according to Method A. Qualitative ninhydrin test positive.

Ar-CO-NH-Cys(Trt)-Rink-PS (2a–t). Carboxylic acids (**3a–t**) were coupled to resin H-Cys(Trt)-Rink-PS (0.42 g, 0.34 mmol) according to Method B.

Ar-CO-NH-Cys-Rink)$_2$-PS (4a–t). Resins (**2a–t**) were treated according to Method C.

Cystine derivatives (1a–t). Resins (**4a–t**) were treated according to Method D.

N,N′-*Di-(*p*-anisoyl)-L-cystine diamide (1a) (10).* Yield: 85%; R_f: 0.47 ($CHCl_3$/MeOH, 9/1); IR (neat): 1660, 1627, 1606, 1502 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.40 (d, 2H, $J = 9$ Hz, N*H*), 7.84 (d, 4H, $J = 9$ Hz, Ar*H*), 7.47 (br s, 2H, 2N*H*H), 7.19 (br s, 2H, 2NH*H*), 6.97 (d, 4H, $J = 9$ Hz, Ar*H*), 4.68 (ddd, 2H, $J = 13$, 9, 5 Hz, Cystine-H_α), 3.80 (s, 6H, CH_3O), 3.24 (dd, 2H, $J = 13$, 5 Hz, Cystine-H_β), 3.06 (dd, 2H, $J = 13$, 10 Hz, Cystine-H_β); ^{13}C-NMR (DMSO-d_6, 100 MHz,): δ 172.2 (*C*ONH$_2$), 165.9 (*C*ONH), 161.7 (*ipso*-Ar*C*), 129.3 (Ar*C*H), 126.2 (*ipso*-Ar*C*), 113.4 (Ar*C*H), 55.3 (O*C*H$_3$), 52.5 (Cystine-C_α), 40.1 (Cystine-C_β); *m/z* (ES$^+$): 507.4 (M + H)$^+$, 529.3 (M + Na)$^+$; RP-HPLC (ELSD): 8.0 min (91%) (Method 1).

N,N′-*Di(2-naphthoyl)-L-cystine diamide (1b) (10).* Yield: 90%; RP-HPLC (λ_{254}): 11.6 min (55%), *m/z* (ES$^+$): 547.1 (M + H)$^+$, 569.1 (M + Na)$^+$ (Method 2).

N,N′-*Di(*p*-nitrobenzoyl)-L-cystine diamide (1c).* Yield: 50%; LCMS (λ_{254}): 9.6 min (100%), *m/z* (ES$^+$): 537.0 (M + H)$^+$, 559 (M + Na)$^+$ (Method 2).

N,N′-*Di(*p*-toluoyl)-L-cystine diamide (1d).* Yield: 61%; LCMS (λ_{254}): complex mixture, *m/z* (ES$^+$): 475.1 (M + H)$^+$, 497.1 (M + Na)$^+$ (Method 2).

N,N′-*Di(benzoyl)-L-cystine diamide (1e).* Yield: 70%; LCMS (λ_{254}): 9.2 min (100%), *m/z* (ES$^+$): 447.0 (M + H)$^+$, 469.0 (M + Na)$^+$, 915 (2M + Na)$^+$ (Method 2).

N,N′-*Di(*p*-bromobenzoyl)-L-cystine diamide (1f).* Yield: 44%; LCMS (λ_{254}): 10.8 min (100%), *m/z* (ES$^+$): 602.8 (M + H)$^+$, 624.8 (M + Na)$^+$ (relative to ^{79}Br, most abundant isotope) (Method 2).

N,N′-*Di(*p*-methylthiobenzoyl)-L-cystine diamide (1g).* Yield: 66%; LCMS (λ_{254}): 10.3 min (100%), *m/z* (ES$^+$): 539.0 (M + H)$^+$, 561.0 (M + Na)$^+$ (Method 2).

N,N′-*Di(*p*-fluorobenzoyl)-L-cystine diamide (1h).* Yield: 57%; LCMS (λ_{254}): 9.7 min (100%), *m/z* (ES$^+$): 483.0 (M + H)$^+$, 505.0 (M + Na)$^+$, 987.0 (2M + Na)$^+$ (Method 2); R_f: 0.44 ($CHCl_3$/MeOH, 9/1); IR (neat): ν_{max}: 1668, 1636, 1602 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.61 (d, 2H, $J = 8.5$ Hz, N*H*), 7.92 (dd, 4H, $J = 9$ Hz, $^4J_{HF} = 5$ Hz, 2Ar*H*-2 + 2Ar*H*-6), 7.52 (br s, 2H, NH_2), 7.26 (t, 4H, $J = 9$ Hz, $^3J_{HF} = 9$ Hz, 2Ar*H*-3 + 2Ar*H*-5), 7.21 (br s, 2H, NH_2), 4.69 (m,

2H, Cystine-H_α), 3.26 (dd, 2H, $J = 13$, 4 Hz, Cystine-H_β), 3.03 (dd, 2H, $J = 13$, 10 Hz, Cystine-H_β); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 172.1 (*C*ONH$_2$), 165.5 (*C*ONH), 164.0 (d, $^1J_{CF} = 248$ Hz, Ar*C*H-4), 130.5 (d, $^4J_{CF} = 3$ Hz, Ar*C*-1), 130.2 (d, $^3J_{CF} = 9$ Hz, Ar*C*H-2 + Ar*C*H-6), 115.1 (d, $^2J_{CF} = 21$ Hz, Ar*C*H-3 + Ar-*C*H-5), 52.6 (Cystine-C_α), 40.0 (Cystine-C_β); m/z (ES$^+$): 483.2 (M + H)$^+$; HRMS (ES$^+$): calcd for $C_{20}H_{21}N_4O_4F_2S_2$(M + H)$^+$, 483.0723, found (M + H)$^+$ 483.0967; RP-HPLC (λ_{254}): 8.2 min (98%) (Method 1).

N,N′-*Di-furanoyl-L-cystine diamide (1i)*. Yield: 73%; LCMS (λ_{254}): 7.74 min (70%) + 7.95 min (30%) mixture of two peaks not resolved on the TIC profile, m/z (ES$^+$): 427.0 (M + H)$^+$, 449.0 (M + Na)$^+$, 875.0 (2M + Na)$^+$ (Method 2).

N,N′-*Di-thiophenoyl-L-cystine diamide (1j)*. Yield: 30%; LCMS (λ_{254}): 8.9 min (100%), m/z (ES$^+$): 459.0 (M + H)$^+$, 481.0 (M + Na)$^+$, 938.9 (2M + Na)$^+$ (Method 2).

N,N′-*Di-pyridynoyl-L-cystine diamide (1k)*. Yield: 38%; LCMS (λ_{254}): 3.4 (91%), m/z (ES$^+$): 225.1 (M + 2H)$^{2+}$, 449.0 (M + H)$^+$ (Method 2).

N,N′-*Di-(p-methylbenzyloyl)-L-cystine diamide (1l)*. Yield: 50%; LCMS (λ_{210}): 10.3 min (64%), m/z (ES$^+$): 525.0 (M + Na)$^+$ (Method 2).

N,N′-*Di(1-naphthoyl)-L-cystine diamide (1m)*. Yield: 21%; LCMS (λ_{254}): 10.7 min (95%), m/z (ES$^+$): 547.0 (M + H)$^+$, 569.0 (M + Na)$^+$ (Method 2).

N,N′-*Di(p-hexylbenzoyl)-L-cystine diamide (1n)*. Yield: 44%; LCMS (λ_{254}): 15.6 min (100%), m/z (ES$^+$): no corresponding peak found (Method 2).

N,N′-*Di(2-naphthyloxyacetyl)-L-cystine diamide (1o)*. Yield: 22%; LCMS (λ_{254}): 11.6 min (90%), m/z (ES$^+$): 607.1 (M + H)$^+$, 629.0 (M + Na)$^+$ (Method 2).

N,N′-*Di-(p-hydroxybenzyloyl)-L-cystine diamide (1p)*. Yield: 52%; LCMS (λ_{254}): complex mixture (Method 2).

N,N′-*Di-(p-chloromethylbenzoyl)-L-cystine diamide (1q)*. Yield: 57%; RP-HPLC (λ_{254}): 10.4 min (100%); m/z (ES$^+$): 565.0, 567.0 (M + Na)$^+$ (Method 2)

N,N′-*Di-(m-cyanobenzoyl)-L-cystine diamide (1r)*. Yield: 41%; RP-HPLC (λ_{254}): 9.2 min (83%) (Method 1); m/z (ES$^+$): 519.0 (M + H)$^+$ (Method 2).

N,N′-*Di-(p-chlorobenzoyl)-L-cystine diamide (1s)*. Yield: 65%; LC-MS (λ_{254}): 9.7 min (79%), m/z (ES$^+$): 515.0 (M + H)$^+$, 537.0 (M + Na)$^+$ (relative to the most abundant ^{35}Cl isotope) (Method 2).

N,N′-*Di-(p-iodobenzoyl)-L-cystine diamide (1t)*. Yield: 86%; LC-MS (λ_{254}): 10.3 min (59%), ES$^+$: 698.9 (M + H)$^+$, 720.9 (M + Na)$^+$ (Method 2).

N-*anisoyl-L-cysteine amide (5a)*. Resin (**2a**) was cleaved according to Method D. RP-HPLC (ELSD): 8.1 min (92%) (Method 2); LC-MS (λ_{254}): 3.8 min (73%), m/z (ES$^+$): 277.1 (M + H)$^+$ (Method 3).

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