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A facile synthesis of 1,2,4-triazine fused pyrroline nitroxides as useful paramagnetic building block

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ABSTRACT

In this study, we report an efficient synthesis of 3-amino- or 3-methylsulfanyl-6-oxyl-pyrrolo[3,4-e][1,2,4]triazine radical from *O*-methyl protected 3,4-dioxopyrrolidine pre-nitroxide. From the amino derivative, the corresponding 3-iodo-6-oxyl-pyrrolo[3,4-e][1,2,4]triazine radical was obtained via radical substitution. This compound was an excellent substrate for Pd-catalyzed cross-coupling reactions. The oxidation of *O*-methyl protected 3-methylsulfanyl-pyrrolo[3,4-e][1,2,4]triazine with excess amount of 3-chloroperbenzoic acid afforded a 3-methylsulfonyl-6-oxyl-pyrrolo[3,4-e][1,2,4]triazine radical that could be used in aromatic nucleophilic substitution reactions to produce new paramagnetically modified amino acids and polycyclic compounds via an intramolecular Diels-Alder reaction.

1. Introduction

The 1,2,4-triazines are important organic scaffolds, and they are widely used as key reaction partners in strain-promoted inverse electron-demand Diels-Alder cycloaddition reactions with *trans*-cyclooctene (TCO) [1,2] and cyclooctyne system [3,4], making them capable of bioorthogonal labeling. The 1,2,4-triazines have been identified in microbial natural products such as Toxoflavin (**1**) isolated from *Pseudomonas cocovenenans* [5] or Fervenulin (**2**) isolated from cultures of *Streptomyces fervens* [6]. The 1,2,4-triazine motif can also be found in many drugs, such as Lamotrigine (**3**) [7], an anticonvulsant, and Remdesivir (**4**), a well-known RNA polymerase inhibitor [8], to name just a few. (Fig. 1). The 1,2,4-triazines are widely used as auxiliary heterocyclic building blocks to construct furo[2,3-*b*]pyridines, dihydropyrano[2,3-*b*]pyridines, and pyrrolo[2,3-*b*]pyridines [9] via intramolecular Diels-Alder reactions. The 1,2,4-triazine methodology is widely used in pyridine synthesis with inverse Diels Alder reactions, e.g., reaction of the electron-deficient 1,2,4-triazine with electron-rich dienophiles, such as enamines or silyl-enolethers [10,11].

To extend the above issues to paramagnetic species, e.g., stable nitroxide free radicals, we continued our research by constructing 1,2,4-triazine-condensed pyrroline nitroxides. We have reported earlier 1,2-, 1,3-, and 1,4-diazine condensed pyrroline nitroxides [12–14], and we proposed that the key starting compound of the latter, 1-methoxy-2,2,5,5-tetramethylpyrrolidine-3,4-dione **5**, as a 1,2-dicarbonyl compound, can be a useful substrate to construct 1,2,4-triazine-fused pyrroline nitroxides according to Erikson's works [15,16]. In the present paper, we report the facile synthesis of (5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazin-6-yl)oxydanyl scaffolds with various substituents mainly in position-3 of the triazine unit to demonstrate their usefulness in the construction of polycyclic paramagnetic compounds, paramagnetic amino acids, SH-specific spin labels, and substrates capable of Pd-catalyzed cross-coupling reactions. The paramagnetic polycyclic compounds were useful spin-probe tools for exploring the internal structure of graphene oxide membranes [17], while paramagnetic L-amino acids could be incorporated into proteins to investigate their structure and function [18,19].

2. Results and discussions

Reaction of dicarbonyl compound **5** with *S*-methyl thiosemicarbazide hydrogen iodide [20] in aq. methanol in the presence of $NaHCO_3$ offered triazine compound **6**. To remove the *O*-methyl group [21] of this compound to access the nitroxide unit upon treatment with 3.0 eq. *m*-CPBA we got a mixture of compounds **7, 8,** and **9** (details see in the SI)**.** To achieve exclusively compound **8** as a useful intermediate,

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compound **6** was subjected to 8.0 eq. *m*-CPBA treatment and an 8 h reaction time, to give compound **8**, as the main product in 72% yield after purification. Reaction of compound **5** with aminoguanidine HCl salt in the presence of $NaHCO_3$ in aq. ethanol at reflux temperature offered compound **10**. Upon treatment of compound **10** with 3.0 eq. *m*-CPBA, we received the mixture of compounds **11** and **12** (see details in the SI). Compound **12** could also be achieved as a sole product by treatment of compound **10** in 68% yield with 5.0 eq. *m*-CPBA in DCM on a prolonged oxidation time (4 h) (Scheme 1.). Compound **12** was crystallized from a DCM/hexane mixture to achieve single crystals for structure elucidation. A single-crystal X-ray investigation unambigously proved it to be the (3-amino-2-oxo-5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazin-6-yl)oxydanyl (Fig. 2).

Compound **10** treatment with isopentyl nitrite in THF via diazotation reaction in a radical substitution reaction [1] offered compound **13**, which was then deprotected with 2.5 eq. *m*-CPBA for (5,5,7,7-tetramethyl-6,7-dihydro-5*H*-pyrrolo[3,4-e][1,2,4]triazine-6-yl)oxydanyl **14**. Reaction of compound **14** with a strained bicyclononyne ester at 37 °C has not provided the expected Diels-Alder adduct [3], even upon prolonged reaction time (~36 h, data not shown). By the methodology of Horner, K. A. et al. [3], treatment of compound **10** with 14.0 eq. isopentyl nitrite in the presence of CH_2I_2 in a radical substitution reaction, we have received iodo compound **15**, which, after treatment with *m*-CPBA, offered paramagnetic iodo compound **16**. In a model reaction, phenylboronic acid ester in the presence of $Pd(PPh_3)_4$ with base in aq. dioxan gave 3-phenyl derivative **17** in 79% yield [22]. The Sonogashira coupling was successful as well; the reaction of the compound **16** with phenylacetylene in the presence of CuI and $PdCl_2(PPh_3)_2$ in THF/Et_3N offered compound **18** in 63% yield, demonstrating that compound **16** is a useful substrate in Pd-catalyzed cross-coupling reactions [23] (Scheme 2).

The sulfone function on an electron-deficient heterocyclic ring in compound **8** was supposed to be a good leaving group in a nucleophilic substitution reaction [11,24]. It was confirmed that treatment with excess NH_3 gas in THF furnished **19** amine compound, which was not accessible by the deprotection of compound **10**. Utilization of the method of Boerema et al. for the synthesis of lysine-heterocycle hybrids [25] N^{α}-(tert-butoxycarbonyl)-L-lysine was treated with compound **8** in the presence of Na_2CO_3 in DMSO at 70 °C overnight, offering spin-labeled L-lysine **20**, which can be incorporated into a protein as a non-canonical paramagnetic amino acid. The sulfone group can also be substituted with *O*-nucleophiles, as with aq. KOH in ethanol, furnishing compound **21.** Based on the IR band at 1666 cm^{-1} and the ^{13}C signal at 156.2 ppm, we propose the lactam form more properly than the hydroxy form. Reaction of compound **8** with homopropargyl alcohol sodium salt in THF afforded compound **22.** This ether was heated in chlorobenzene at reflux temperature for 40 h to furnish (5,5,7,7-tetramethyl-2,3,5,7-tetrahydro-6*H*-furo[2,3-*b*]pyrrolo[3,4-e]pyridin-6-yl)oxydanyl tricyclic compound **23** in an intramolecular Diels-Alder reaction, in a low 28% yield [9]. Our attempts to aromatize the dihydrofuran ring using DDQ were unsuccessful. We were inspired by the work of Yao G. et al. [26], conducting spin labeling with a pyrimidine sulfone attached trytyl radical, we propose that compound **8** can be used directly as a spin label too. We were pleased to observe that in a model reaction, compound **8** with *N*-acetylcysteine methyl ester [27] reacted in MeOH in the presence of Et_3N within 15 min., providing isolable adduct **24** in 73% and proving that cysteine labeling is possible. This reaction was extended to N^{α}-(tert-butoxycarbonyl)-*L*-cysteine and again, in mild reaction conditions, offered a cysteine-based, paramagnetic, peptide-incorporable non-canonical alpha-amino acid **25** (Scheme 3).

3. Conclusions

In summary, this study successfully developed an efficient synthesis of paramagnetic (3-substituted-5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazine-6-yl)oxydanyl radicals, which proved to be versatile paramagnetic key compounds. These can be converted to spin labels, paramagnetic amino acids, iodo derivatives for Pd-catalyzed cross-coupling reactions, and nitroxide-ring containing polycyclic compounds. We have also explored the scope and limitations of deprotecting NOMe groups to furnish nitroxide moieties in the presence of sulfides and other heterocyclic ring nitrogens in the molecule. Moving forward, we plan to conduct classical (cysteine spin labeling) by compound **8** and a biorthogonal spin labeling by compound **14** with a more reactive [28] *trans*-cyclooctene-containing amino acid side-chain-incorporated peptide.

3.1. Experimental general

Melting points were determined with a Boetius and Cole-Parmer MP400 micro-melting point apparatus and were uncorrected. Elemental analyses (C, H, N, and S) were performed with a Fisons EA 1110 CHNS elemental analyzer. Mass spectra were recorded on a Shimadzu GCMS-QP2020 spectrometer with EI (70 eV) and a Thermo

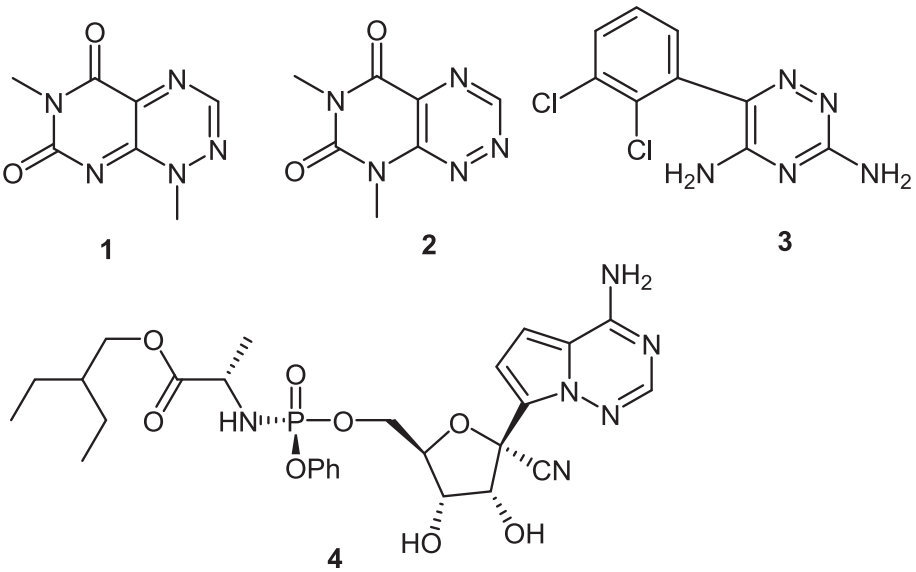
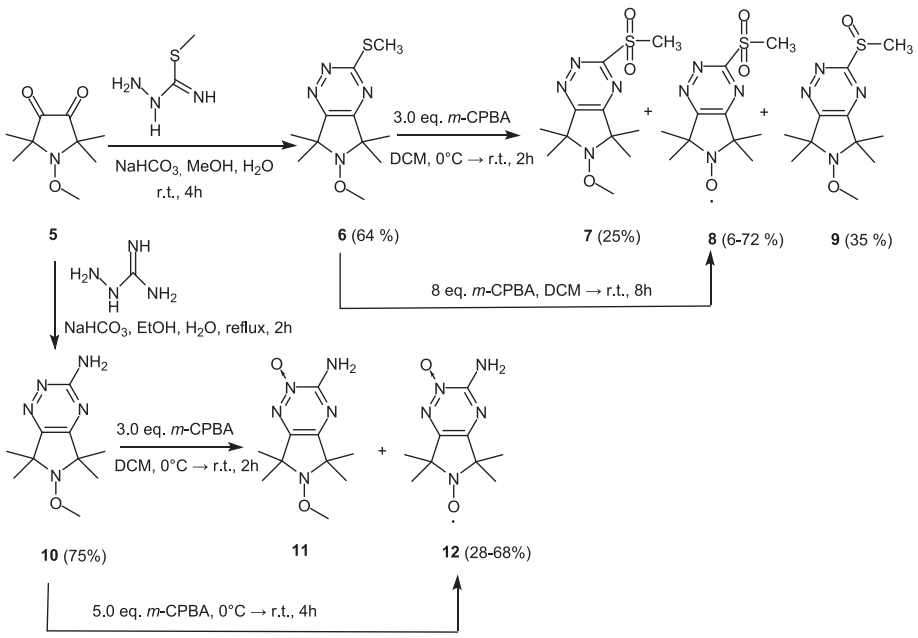


Fig. 1. 1,2,4 Triazine-ring-containing bioactive molecules and drugs.



Scheme 1. Synthesis of diamagnetic and paramagnetic 5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazine scaffolds

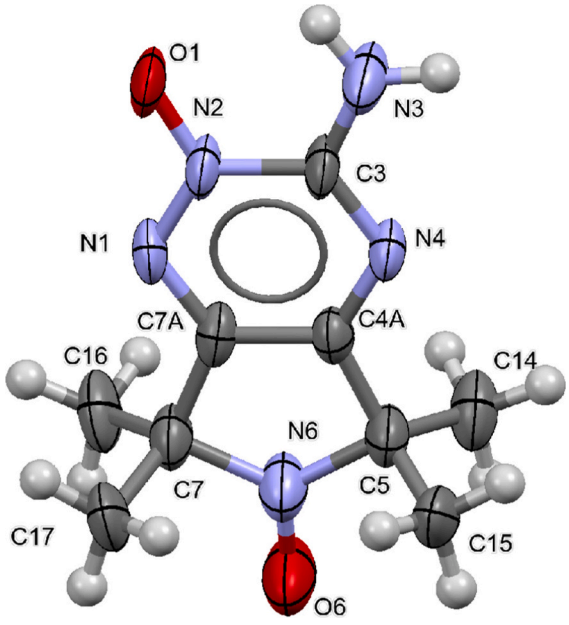


Fig. 2. ORTEP view of the structure of compound **12** at 50% probability level with numbering scheme.

Scientific Q-Exactive HPLC/MS/MS with ESI(+) ionization. NMR spectra were recorded on a Bruker Avance III Ascend 500 spectrometer; chemical shifts are referenced to TMS. The paramagnetic compound was reduced to *N*-hydroxylamines using five equivalents of hydrazobenzene (DPPH) radical. Measurements were performed at a probe temperature 298 K in $CDCl_3$ or d_6-DMSO solution. ESR spectra were recorded on Miniscope MS 200 in $CHCl_3$ solution. All monoradicals gave a triplet line at 13.9–14.4 G. The total irradiation time was as indicated. IR spectra were recorded with a Bruker Alpha FT-IR instrument with ATR support (diamond plate). Flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). Diffraction intensity data were collected at room temperature using a Bruker-D8 Venture diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) equipped with INCOATEC IμS 3.0 (Incoatec GmbH, Geesthacht, Germany) dual (Cu and Mo) sealed tube micro sources and a Photon II Charge-Integrating Pixel Array detector (Bruker AXS GmbH, Karlsruhe, Germany) using Cu Kα (λ = 1.541 Å) radiation, for further details see the SI.

Compound **5** [14], *S*-methyl thiosemicarbazide hydrogen iodide [20], and *N*-acetylcysteine methyl ester [27] were prepared as described previously. The *m*-CPBA was prepared, and concentration was monitored as reported earlier [29]. Other reagents and solvents were purchased from Merck or OQEMA.

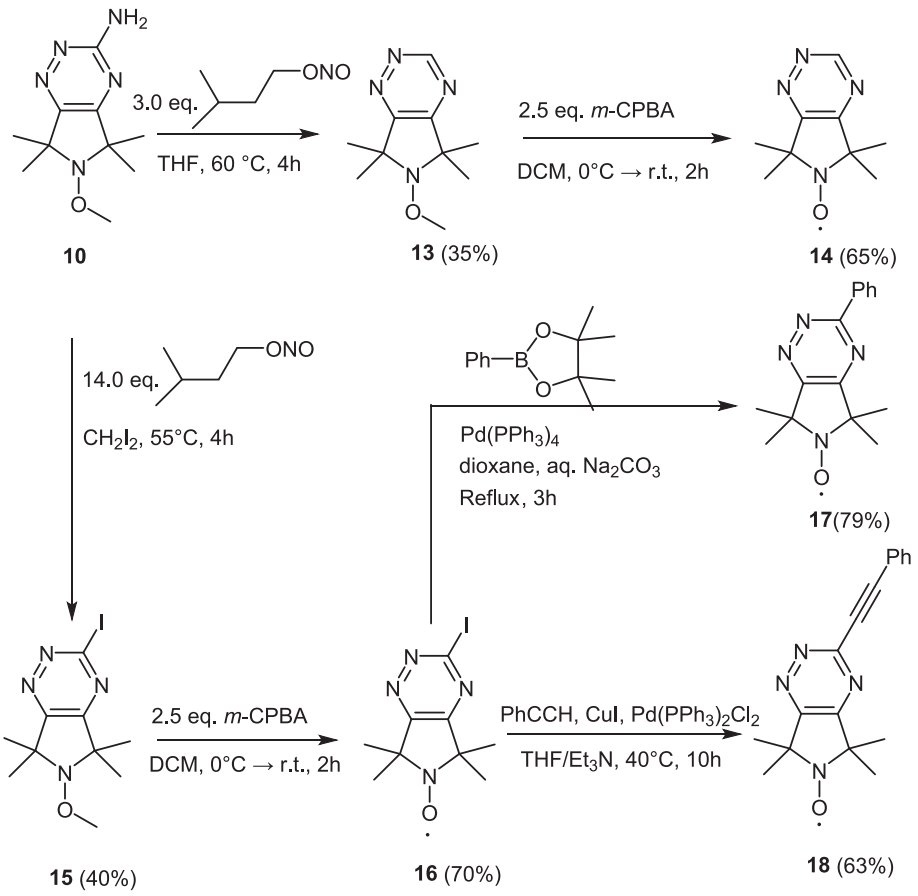
6-Methoxy-5,5,7,7-tetramethyl-3-(methylthio)-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazine (6):

A solution of compound **5** (3.70 g, 20.0 mmol), *S*-methyl thiosemicarbazide hydrogen iodide (4.89 g, 21.0 mmol), and $NaHCO_3$ (2.10 g, 25.0 mmol) in MeOH (40 mL) and water (10 mL) was stirred at 25 °C for 4 h. Then the MeOH was evaporated under reduced pressure; the residue was diluted with brine (20 mL), and the reaction mixture was extracted with EtOAc (2 × 20 mL). The combined organic phase was dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by flash column chromatography to give the title compound 3.1 g (64%) as a white solid, mp 60–61 °C, R_f: 0.66 (hexane/EtOAc, 2:1). IR (ν/cm^{-1}): 2971, 2929, 2852, 2809, 1572, 1529. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.49 (6H, s, C(C*H*$_3$)$_2$), 1.57 (6H, s, C(C*H*$_3$)$_2$), 2.68 (3H, s, SC*H*$_3$), 3.80 (3H, s, OC*H*$_3$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 14.0 (1C), 22.8 (2C), 27.7 (2C), 65.3 (1C), 65.7 (1C), 65.8 (1C), 159.8 (1C), 164.9 (1C), 173.1 (1C). MS (EI): *m/z* (%) = 254 (M^+, 31), 239 (100), 209 (8), 138 (62).

Anal. Calcd for $C_{11}H_{18}N_4OS$: C, 51.94; H, 7.13; N, 22.03; S, 12.60; Found: C, 51.84; H, 7.31; N, 21.93; S, 12.62.

(5,5,7,7-Tetramethyl-3-(methylsulfonyl)-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (8):

To a stirred solution of compound **6** (2.54 g, 10.0 mmol) in CH_2Cl_2 (40 mL), 3-chloroperbenzoic acid (17.75 g (77%), 80.0 mmol) was added in 10 portions during 2 h at 0 °C, then the reaction was allowed to warm to 25 °C and stirred for a further 6 h. The precipitate was filtered out through a glass frit and washed with CH_2Cl_2 (5 mL). The combined organic phase was washed with 10% aq. Na_2CO_3 (2 × 30 mL) and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was subjected to flash column chromatography (hexane/EtOAc to elute compounds **8** as a pink solid 1.95 g (72%), mp 190–191 °C. IR (ν/cm^{-1}): 3018, 2989, 2979, 2935, 1663, 1680, 1569, 1543. ^{1}H NMR (DMSO-d_6, +(PhNH)$_2$, 500 MHz): δ = 1.58 (6H, s, C(C*H*$_3$)$_2$), 1.64 (6H, s, C(C*H*$_3$)$_2$),



Scheme 2. Radical reactions of 3-amino-6-methoxy-5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazine and Pd-catalized cross-coupling reaction of (3-iodo-5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo[3,4-e][1,2,4]triazine-6-yl)oxydanyl

3.54 (3H, s, SO_2CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 24.2 (2C), 24.7 (2C), 39.9 (1C), 65.9 (1C), 66.4 (1C), 166.8 (1C), 167.0 (1C), 168.4 (1C). MS (EI): *m/z* (%) = 271 (M^+, 22), 241 (3), 134 (12), 108 (100). Anal. Calcd for $C_{10}H_{15}N_4O_3S$: C, 44.27; H, 5.57; N, 20.65; S, 11.82; Found: C, 44.12; H, 5.61; N, 20.58; S, 11.72.

3.2. 3-Amino-6-methoxy-5,5,7,7-tetramethyl-6,7-dihydro-6H-pyrrolo [3,4-e][1,2,4]triazine (10):

A solution of compound **5** (3.70 g, 20.0 mmol), aminoguanidine hydrochloride (2.30 g, 21.0 mmol), and $NaHCO_3$ (2.10 g, 25.0 mmol) in EtOH (40 mL) and water (10 mL) was heated at reflux temperature for 2 h. Then the ethanol was evaporated off in a vacuum, the residue was diluted with brine (20 mL), and the reaction mixture was extracted with EtOAc (2 × 20 mL). The combined organic phase was dried ($MgSO_4$), filtered and evaporated and the residue was purified by flash column chromatography to give the title compound 3.0 g (75%) as a white solid, mp 228–229 °C, R_f: 0.33 (hexane/EtOAc, 1:1). IR (ν/cm^{-1}): 3330, 3316, 3146, 2973, 2934, 1652, 1588, 1538. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.45 (6H, s, $C(CH_3)_2$), 1.53 (6H, s, $C(CH_3)_2$), 3.78 (3H, s, OCH_3), 5.80 (2H, bs, NH_2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 21.2 (2C), 28.5 (2C), 65.0 (1C), 65.6 (1C), 65.7 (1C), 155.3 (1C), 162.6 (1C), 167.2 (1C). MS (EI): *m/z* (%) = 223 (M^+, 1), 208 (10), 178 (19), 150 (5), 108 (100).

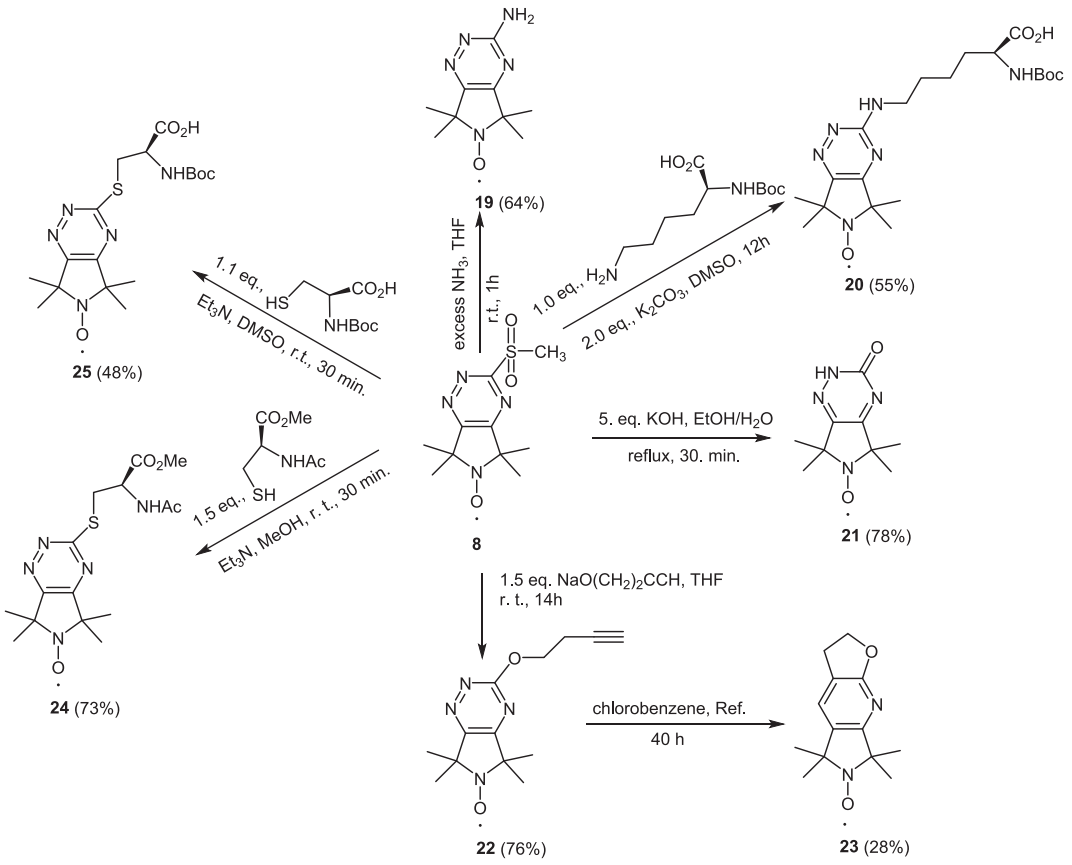
Anal. Calcd for $C_{10}H_{17}N_5O$: C, 53.79; H, 7.67; N, 31.37; Found: C, 53.72; H, 7.37; N, 31.15.

(3-Amino-2-oxo-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (12): To a stirred solution of compound **10** (2.23 g, 10.0 mmol) in CH_2Cl_2 (40 mL), 3-chloroperbenzoic acid (12.3 g (70%), 50.0 mmol) was added in 6 portions over 1 h. at 0 °C, then the reaction was allowed to warm to 25 °C and stirred for a further 3 h. The precipitate was filtered out through a glass frit and washed with CH_2Cl_2 (5 mL). The combined organic phase was washed with 10% aq. Na_2CO_3 (2 × 25 mL) and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was subjected to flash column chromatography (hexane/EtOAc) to eluate compounds **12**, 1.52 g (68%). Rf: 0.16 (hexane/EtOAc, 2:1) was isolated as a deep yellow crystalline solid, mp 185–186 °C. IR: (ν/cm^{-1}): 3338, 3292, 3206, 3132, 2983, 2935, 1641, 1535. ^{1}H NMR ($CDCl_3$, + $(PhNH)_2$) 500 MHz): δ = 1.63 (6H, s, $C(CH_3)_2$), 1.68 (6H, s, $C(CH_3)_2$), 6.32 (2H, bs, NH_2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 23.9 (2C), 24.5 (2C), 66.7 (1C), 67.5 (1C), 148.9 (1C), 151.21 (1C), 151.23 (1C). MS (EI): *m/z* (%) = 224 (M^+, 23), 194 (61), 164 (12), 108 (100). Anal. Calcd for $C_9H_{14}N_5O_2$: C, 48.21; H, 6.29; N, 31.23; Found: C, 48.07; H, 6.19; N, 31.08.

For single crystal X-ray, compound **12** (150 mg) was dissolved in CH_2Cl_2 (2 mL) in a test tube, and hexane was added till turbidity, and allowed to stay at 25 °C for several days.

6-Methoxy-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazine (13):

To a 30 mL pressure tube, a solution of compound **10** (892 mg, 4.0 mmol) in THF (10 mL) and isopentyl nitrite (1.6 mL, 12.0 mmol) was added. The tube was flushed with nitrogen, sealed, and heated at 60 °C for 4 h. The crude product was gently concentrated in vacuo and purified by flash column chromatography eluting with Hexane/EtOAc to provide compound **13,** 291 mg (35%) as a yellow oil R_f: 0.33 (hexane/EtOAc 1:1). IR (ν/cm^{-1}): 2979, 2935, 1635, 1551. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.52 (6H, s, $C(CH_3)_2$), 1.61 (6H, s, $C(CH_3)_2$), 3.83 (3H, s, OCH_3), 9.49 (1H, s, Ar*H*). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 23.0 (2C), 29.5 (2C), 65.2 (1C), 65.8 (1C), 66.0 (1C), 157.2 (1C), 164.3 (1C), 162.2 (1C). MS (EI): *m/z* (%) = 208 (M^+, 15), 193 (100), 162 (50). Anal. Calcd for $C_{10}H_{16}N_4O$: C, 57.67; H, 7.74; N, 26.90; Found: C, 57.55; H, 7.70; N,



Scheme 3. Nucleophilic substitution reactions of (5,5,7,7-tetramethyl-3-(methylsulfonyl)-5,7-dihydro-6H-pyrrolo[3,4-e][1,2,4]triazin-6-yl)oxydanyl

26.78.

(5,5,7,7-Tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (14):

To a stirred solution of compound **13** (312 mg, 1.5 mmol) in CH_2Cl_2 (20 mL), 3-chloroperbenzoic acid (921 mg (70%), 3.75 mmol) was added in 2 portions over 20 min. at 0 °C, then the reaction was allowed to warm to 25 °C and stirred further with continuous monitoring with TLC. After consumption of the starting material (~1 h), the organic phase was washed with 10% aq. Na_2CO_3 (2 × 10 mL) and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was subjected to flash column chromatography (hexane/EtOAc to elute compound 188 mg (65%) **14** as a yellow solid**,** mp 83–84 °C, R_f: 0.60 ($CHCl_3$/Et_2O, 2:1).

IR (ν/cm^{-1}): 3067, 2980, 2934, 1574, 1548. ^{1}H NMR ($CDCl_3$, + $(PhNH)_2$ 500 MHz): δ = 1.62 (6H, s, C(C*H*$_3$)$_2$), 1.72 (6H, s, C(C*H*$_3$)$_2$), 9.60 (1H, s, Ar*H*). ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): δ = 24.0 (2C), 24.6 (2C), 67.4 (1C), 67.5 (1C), 157.6 (1C), 163.5 (1C), 164.1 (1C). MS (EI): *m/z* (%) = 193 (M^+, 20), 179 (9), 156 (18), 108 (100), 93 (58). Anal. Calcd for $C_9H_{13}N_4O$: C, 55.94; H, 6.78; N, 29.00; Found: C, 56.01; H, 6.68; N, 28.95.

3-Iodo-6-methoxy-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazine (15):

To a 30 mL pressure tube, a mixture of compound **10** (892 mg, 4.0 mmol), isopentyl nitrite (7.5 mL, 56.0 mmol), and CH_2I_2 (5 mL, 61.5 mmol) was added. The tube was flushed with nitrogen, sealed, and the two-phase mixture was vigorously stirred and heated at 55 °C for 4 h. The crude product was concentrated in vacuo as far as possible to leave the product and unreacted diidomethane (ca. 4 mL). The residue was dried on silica gel directly (~ 20 g) and purified by flash column chromatography on (~50 g silica gel column) eluting with hexane first, to get rid of unreacted diidomethane, then with (hexane/Et_2O, 10:1) to furnish the title product 534 mg (40%) **15** as an off-white solid, mp: 92–93 °C, R_f: 0.69 (hexane/Et_2O 2:1). IR (ν/cm^{-1}): 2971, 2928, 2807, 1552, 1523. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.50 (6H, s, C(C*H*$_3$)$_2$), 1.59 (6H, s, C(C*H*$_3$)$_2$), 3.79 (3H, s, OC*H*$_3$). ^{13}C NMR ($CDCl_3$ 125 MHz): δ = 22.4 (2C), 23.7 (2C), 65.4 (1C), 65.80 (1C), 65.84 (1C), 133.8 (1C), 163.2 (1C), 167.2 (1C). MS (EI): *m/z* (%) = 334 (M^+, 7), 319 (100), 288 (15), 138 (36). Anal. Calcd for $C_{10}H_{15}IN_4O$: C, 35.94; H, 4.52; N, 16.77; Found: C, 35.82; H, 4.35; N, 16.59.

(3-Iodo-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (16):

To a stirred solution of compound **15** (668 mg, 2.0 mmol) in CH_2Cl_2 (20 mL), 3-chloroperbenzoic acid (1.32 g (65%), 5.0 mmol) was added in 2 portions over 20 min. at 0 °C, then the reaction was allowed to warm to 25 °C and stirred for further with continuous monitoring with TLC. After consumption of the starting material (~2 h), the organic phase was washed with 10% aq. Na_2CO_3 (1 × 10 mL) and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was subjected to flash column chromatography (hexane/Et_2O) to elute compound **16**, 446 mg (70%) as a red solid**,** mp 109–110 °C, R_f: 0.62 (hexane/EtOAc, 2:1). IR (ν/cm^{-1}): 2981, 2931, 1562,1522. ^{1}H NMR ($CDCl_3$,+$(PhNH)_2$, 500 MHz): δ = 1.54 (6H, s, C(C*H*$_3$)$_2$), 1.63 (6H, s, C(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): δ = 24.0 (2C), 24.6 (2C), 65.9 (1C), 66.2 (1C), 162.9 (1C), 166.7 (1C). The *C*—I signal overlaps with signals of $(PhNH)_2$. During the long ^{13}C measurement time, compound **16** reacted with $(PhNH)_2$ to produce diverse minor peaks in the aromatic region. MS (EI): *m/z* (%) = 319 (M^+, 7), 194 (10), 108 (100). Anal. Calcd for $C_9H_{12}IN_4O$: C, 33.87; H, 3.79; N, 17.56; Found: C, 33.80; H, 3.73; N, 17.42.

(5,5,7,7-Tetramethyl-3-phenyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (17):

To a stirred, deoxygenated solution of compound **16** (319 mg, 1.0 mmol) in dioxane (10 mL), $Pd(PPh_3)_4$ (57 mg, 0.05 mmol) was added, and the mixture was stirred for 10 min. After adding phenylboronic acid

pinacol ester (224 mg, 1.1 mmol) and 10% aq. Na_2CO_3 solution (5 mL), the mixture was stirred and heated under reflux temperature for 3 h under N_2 atmosphere. After cooling, the solvent was evaporated off, and the residue was partitioned between brine (10 mL) and EtOAc (20 mL). The organic phase was dried ($MgSO_4$), activated MnO_2 (43 mg, 0.5 mmol) was added, and O_2 was bubbled through for 10 min. The mixture was filtered, evaporated, and the residue was subjected to flash column chromatography purification to offer the tile compound **17,**212 mg (79%), deep yellow solid, mp 118–120 °C, R_f: 0.58 (hexane/EtOAc 2:1). IR (ν/cm^{-1}): 2974, 2923, 2852, 1576, 1538. ^{1}H NMR ($CDCl_3$,+$(PhNH)_2$, 500 MHz): $\delta = 1.60$ (6H, s, C(CH_3)$_2$), 1.68 (6H, s, C(CH_3)$_2$), 8.62 (2H, d, $J = 3.5$ Hz, Ar*H*). 3 Ar*H* overlaps with $(PhNH)_2$ signals. ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): $\delta = 24.3$ (2C), 24.9 (2C), 65.5 (1C), 65.9 (1C), 66.2 (1C), 128.3 (2C), 128.8 (2C), 131.4 (1C), 135.3 (1C), 161.9 (1C), 163.9 (1C), 165.2 (1C). MS (EI): m/z (%) = 269 (M^+, 100), 239 (13), 207 (26), 138 (64). Anal. Calcd for $C_{15}H_{17}N_4O$: C, 66.89; H, 6.36; N, 20.80; Found: C, 66.80; H, 6.44; N, 20.72.

(5,5,7,7-Tetramethyl-3-(phenylethynyl)-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (18):

To a stirred, deoxygenated solution of compound **16** (319 mg, 1.0 mmol) in THF (10 mL) and Et_3N (3 mL), $PdCl_2(PPh_3)_2$ (10 mg, 0.014 mmol) and CuI (7.6 mg, 0.04 mmol) were added, and stirring was continued for 10 min. After adding phenylacetylene (112 mg, 1.1 mmol), the mixture color turned brown, and the mixture was stirred at 40 °C for 10 h under N_2 atmosphere. After cooling, the solvent was evaporated off, and the residue was partitioned between brine (10 mL) and EtOAc (20 mL). The organic phase was dried ($MgSO_4$), activated MnO_2 (43 mg, 0.5 mmol) was added, and O_2 was bubbled through for 15 min. The mixture was filtered, evaporated, and the residue was subjected to flash column chromatography purification to offer the title compound **18,**184 mg (63%), orange solid, mp 127–128 °C, $R_f = 0.58$ (hexane/EtOAc, 2:1). IR (ν/cm^{-1}): 2981, 2922, 2851, 2223,1569, 1533. ^{1}H NMR ($CDCl_3$,+$(PhNH)_2$, 500 MHz): $\delta = 1.60$ (6H, s, C(CH_3)$_2$), 1.68 (6H, s, C(CH_3)$_2$), 7.44–7.47 (3H, m, Ar*H*), 7.79 (2H, d, $J = 4.0$ Hz, Ar*H*). ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): $\delta = 24.2$ (2C), 24.8 (2C), 65.9 (1C), 66.2 (1C), 86.1 (1C), 91.8 (1C), 121.0 (1C),128.6 (2C), 130.1 (1C), 132.7 (2C), 154.5 (1C), 161.4 (1C), 164.8 (1C). MS (EI): m/z (%) = 293 (M^+, 9), 233 (84), 203 (100), 188 (49). Anal. Calcd for $C_{17}H_{18}N_4O$: C, 69.37; H, 6.16; N, 19.03; Found: C, 69.30; H, 6.04; N, 18.97.

(3-Amino-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (19):

In a well-ventilated hood, to a stirred solution of compound **8** (1.35 g, 5.0 mmol) in anhydr. THF (40 mL) dry ammonia gas was bubbled through at 0 °C. Upon this, the solution color changed from brown-pink to yellow, and TLC showed the consumption of the starting material (~30 min.). The solution was evaporated off, the residue was dissolved in CH_2Cl_2 (30 mL), filtered, and the filtrate was washed with 10% aq. Na_2CO_3 (15 mL), brine (20 mL), the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was subjected to flash column chromatography to give compound **19,**665 mg (64%) as a yellow solid, 233–234 °C, $R_f = 0.64$ ($CHCl_3$/Et_2O/MeOH, 8:3:1). IR (ν/cm^{-1}): 3354, 3319, 3163, 2982, 1648, 1588, 1532. ^{1}H NMR (DMSO-d_6,+$(PhNH)_2$, 500 MHz): $\delta = 1.31$ (6H, s, C(CH_3)$_2$), 1.38 (6H, s, C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6 + $(PhNH)_2$, 125 MHz): $\delta = 24.4$ (2C), 25.4 (2C), 64.2 (1C), 64.8 (1C), 154.2 (1C), 164.4 (1C), 165.7 (1C). MS (EI): m/z (%) = 208 (M^+, 7), 178 (19), 108 (100). Anal. Calcd for $C_9H_{15}N_5O$: C, 51.66; H, 7.23; N, 33.47; Found: C, 51.52; H, 7.14; N, 33.37.

N^2-(tert-butoxycarbonyl)-N^6-(6-oxyl-5,5,7,7-tetramethyl-6,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-3-yl)-L-lysine (20):

The mixture of compound **8** (1.08 g, 4.0 mmol), N^{α}-(t-butoxycarbonyl)-L-lysine (984 mg, 4.0 mmol), and K_2CO_3 (1.10 g, 8.0 mmol) was stirred in DMSO (20 mL) at 70 °C for 12 h. The mixture was poured into a mixture of water (200 mL) and 10% aq. Na_2CO_3 (50 mL). The mixture was extracted with EtOAc (2 × 50 mL) to remove the excess sulphoxide. The pH = 4 of the aq. solution was adjusted with 1 M HCl, and extracted with EtOAc (3 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered and evaporated and after purification ($CHCl_3$/Et_2O then $CHCl_3$/MeOH), we received compound **20,**961 mg, (55%) as a yellow solid, mp 101–102 °C, R_f 0.35 ($CHCl_3$/Et_2O/MeOH, 8:3:1). IR (ν/cm^{-1}): 3326, 2978, 2931, 1701, 1573, 1540. ^{1}H NMR (DMSO-d_6,+$(PhNH)_2$, 500 MHz): $\delta = 1.30$ (6H, s, C(CH_3)$_2$), 1.37 (17H, bs, C(CH_3)$_2$ + C(CH_3)$_3$, +CH_2CH_2CH_2), 1.58–1.70 (4H, m, (CH_2)$_2$), 3.34 (2H, m, NHCH_2), 3.91 (1H, m, C*H*NHCO). ^{13}C NMR (DMSO-d_6 + $(PhNH)_2$, 125 MHz): $\delta = 23.6$ (1C), 24.3 (2C), 25.4. (2C), 28.6 (1C), 28.7 (3C), 31.0 (1C), 41.0 (1C), 53.9 (1C), 64.2 (1C), 64.8 (1C), 78.4 (1C), 154.2 (1C), 155.0 (1C), 165.5 (1C), 165.6 (1C), 174.6(1C). HRMS: (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{34}N_6O_5$ 438.2591, found 438.2572. Anal. Calcd for $C_{20}H_{33}N_6O_5$ C, 54.90; H, 7.60; N, 19.21; Found: C, 54.88; H, 7.51; N, 19.25.

(3-Oxo-5,5,7,7-tetramethyl-2,5,6,7-tetrahydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxidanyl (21):

To a solution of compound **8** (542 mg, 2.0 mmol) in EtOH (10 mL), KOH 560 mg, 10.0 mmol) in water (10 mL) was added, and the mixture was heated at reflux temperature for 30 min. After cooling, the solvents were evaporated off, the residue was taken up in water, and pH = 4 was adjusted with 1 M HCl. The reaction mixture was extracted with EtOAc (3 × 15 mL), the combined organic phase was dried ($MgSO_4$), filtered and evaporated and the residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to yield compound **21,**326 mg (78%) as a pink solid, mp 184–185 °C, R_f = 0.6 ($CHCl_3$/Et_2O/MeOH, 8:3:1). IR (ν/cm^{-1}): 3226, 3158, 3068, 2934, 1666, 1633, 1570. ^{1}H NMR (DMSO-d_6,+$(PhNH)_2$, 500 MHz): $\delta = 1.52$ (6H, s, C(CH_3)$_2$), 1.56 (6H, s, C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6 + $(PhNH)_2$, 125 MHz): $\delta = 23.5$ (2C), 24.3 (2C), 64.9 (1C), 67.0 (1C), 147.3 (1C), 148.5 (1C), 156.2 (1C). MS (EI): m/z (%) = 209 (M^+, 15), 195 (16), 179 (15), 137 (100), 108 (29). Anal. Calcd for $C_9H_{13}N_4O_2$: C, 51.67; H, 6.26; N, 26.78; Found: C, 51.70; H, 6.41; N, 26.70.

(3-(But-3-yn-1-yloxy)-5,5,7,7-tetramethyl-5,7-dihydro-6*H*-pyrrolo [3,4-e] [1,2,4] triazin-6-yl)oxydanyl (22):

To a stirred solution of sodium but-3-yn-1-olate 7.5 mmol (generated from 525 mg but-3-yn-1-ol and 180 mg NaH) in anhydr. THF (20 mL), compound **8** (1.35 g, 5.0 mmol) was added as a solid all at once at 0 °C and allowed to warm to 25 °C and stirred at this temperature for 14 h with exclusion of moisture. A saturated solution of Na_2CO_3 was then added (15 mL), and the mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phase was dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **22,**991 mg, (76%), 114–115 °C, $R_f = 0.55$ (hexane/EtOAc 2:1). IR (ν/cm^{-1}): 3238, 2985, 2935, 1599, 1545. ^{1}H NMR ($CDCl_3$ + $(PhNH)_2$, 500 MHz): $\delta = 1.53$ (6H, s, C(CH_3)$_2$), 1.61 (6H, s, C(CH_3)$_2$), 2.11 (1H, s, ≡C*H*), 2.86 (2H, m, ≡CCH_2), 4.73 (2 h, t, $J = 7$ Hz, OCH_2). ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): $\delta = 19.1$ (1C), 24.1 (2C), 24.9 (2C), 65.1 (1C), 65.6(1C), 66.6 (1C), 70.3 (1C), 79.9 (1C), 159.5 (1C), 165.5 (1C), 168.5 (1C). MS (EI): m/z (%) = 261 (M^+, 5), 231 (4), 108 (100),

93 (23). Anal. Calcd for $C_{13}H_{17}N_4O_2$: C, 59.76; H, 6.56; N, 21.44; Found: C, 59.69; H, 6.36; N, 21.31.

(5,5,7,7-Tetramethyl-2,3,5,7-tetrahydro-6*H*-furo [2,3-*b*] pyrrolo [3,4-e] pyridin-6-yl)oxydanyl (23): A solution of compound **22** (522 mg, 2.0 mmol) in chlorobenzene (7 mL) was heated at reflux temperature (132 °C) for 40 h. After cooling, the solvent was evaporated off, the residue was subjected to column chromatography (hexane/EtOAc) to furnish the intramolecular D-A adduct **23** as a pale yellow solid 130 mg (28%), mp 138–140 °C, R_f 0.24 (hexane/EtOAc, 2:1). IR (ν/cm^{-1}): 2978, 2924, 2851, 1676, 1591. ^{1}H NMR ($CDCl_3$ + $(PhNH)_2$, 500 MHz): δ = 1.56 (6H, s, C(CH_3)$_2$), 1.60 (6H, s, C(CH_3)$_2$), 3.23 (2H, t, $J = 8.5$ Hz, CH_2), 4.66 (2H, t, $J = 8.5$ Hz, OCH_2), 7.24 (1H, s, Ar*H*). ^{13}C NMR ($CDCl_3$ + $(PhNH)_2$, 125 MHz): $\delta = 24.7$ (2C), 26.2 (2C), 28.0 (1C), 68.2(1C), 69.4 (1C), 69.5 (1C), 119.9 (1C), 127.8 (1C), 129.8 (1C), 159 (1C), 169.3 (1C). MS (EI): m/z (%) = 233 (M^+, 22), 218 (34), 203 (100), 188 (64). Anal. Calcd for $C_{13}H_{17}N_2O_2$: C, 66.93; H, 7.35; N, 12.01; Found: C, 66.75; H, 7.26; N, 11.96.

3.3. Methyl N-acetyl-S-(6-oxyl-5,5,7,7-tetramethyl-6,7-dihydro-5H-pyrrolo[3,4-e][1,2,4]triazin-3-yl)-L-cysteinate (24)

To a stirred solution of *N*-acetyl-*L*-cysteine methyl ester (531 mg, 3.0 mmol) and Et_3N (303 mg, 3.0 mmol) in MeOH (10 mL), compound **8** (542 mg, 2.0 mmol) was added as a solid all at once at 25 °C. The color of the solution changed from pink to yellow, and TLC showed the consumption of compound **8** after 15 min. The solvent was evaporated off, and the residue was partitioned between brine (10 mL) and EtOAc (20 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated to furnish **24** cysteine adduct 537 mg (73%) as a yellow solid after flash column chromatography (hexane/EtOAc), mp 121–122 °C, R_f 0.6 ($CHCl_3$/Et_2O, 2:1). IR (ν/cm^{-1}): 3271, 2976, 1736, 1649,1578, 1545, 1525. ^{1}H NMR (DMSO-d_6 + $(PhNH)_2$, 500 MHz): δ = 1.33 (3H, s, C(CH_3)(CH_3)), 1.38 (3H, s, C(CH_3)(CH_3)), 1.40 (3H, s, C(CH_3)(CH_3)), 1.46 (3H, s, C(CH_3)(CH_3)), 1.85 (3H, s, CH_3CO), 3.40 (1H, m, CH_aH_b), 3.65 (3H, s, OCH_3), 3.84 (1H, m, CH_aH_b), 4.75 (1H, s, C*H*), 8.57 (1H, bs, N*H*). ^{13}C NMR (DMSO-d_6 + $(PhNH)_2$, 125 MHz): δ = 22.7 (1C), 24.3 (2C), 25.0 (2C), 32.0 (1C), 51.7 (1C), 52.6 (1C), 64.6 (1C), 65.2 (1C), 161.1 (1C), 165.9 (1C), 169.9 (1C), 171.4 (1C), 171.6 (1C).

MS (EI): *m/z* (%) = 368 (M^+, 2), 338 (1), 294 (2), 253 (3), 108 (100), 43 (61). Anal. Calcd for $C_{15}H_{22}N_5O_4S$: C, 48.90; H, 6.02; N, 19.01 S, 8.70; Found: C 48.77; H, 5.88; N, 18.91; S, 8.66.

***N*-(tert-butoxycarbonyl)-*S*-(6-oxyl-5,5,7,7-tetramethyl-6,7-dihydro-5*H*-pyrrolo [3,4-e] [1,2,4] triazin-3-yl)-*L*-cysteine (25):**

To a stirred solution of N^{α}-(*tert*-butoxycarbonyl)-L-cysteine (486 mg, 2.2 mmol) and Et_3N (222 mg, 2.2 mmol) in DMSO (5 mL), compound **8** (542 mg, 2.0 mmol) was added as a solid all at once at 25 °C. The color of the solution changed from pink to yellow, and TLC showed the consumption of compound **8** after 30 min. The mixture was poured into a mixture of water (100 mL) and 10% aq. Na_2CO_3 (20 mL). The mixture was extracted with EtOAc (2 × 10 mL) to remove the excess sulphoxide. The pH = 4 of the aq. solution was adjusted with 1 M HCl, and extracted with EtOAc (3 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered and evaporated and after purification ($CHCl_3$/Et_2O then $CHCl_3$/MeOH), we received compound **25,**395 mg (48%) as a yellow solid, mp (166 °C (dec.), R_f 0.32 ($CHCl_3$/MeOH, 5:1). IR (ν/cm^{-1}): 3285, 2974, 2933, 1750, 1695, 1544. ^{1}H NMR (DMSO-d_6,+ $(PhNH)_2$, 500 MHz): δ = 1.35 (9H, s, C(CH_3)$_3$), 1.37 (6H, s, C(CH_3)$_2$), 1.44 (6H, s, C(CH_3)$_2$), 3.40 (1H, m, CH_aH_b), 3.90 (1H, m, CH_aH_b), 4.42 (1H, bs, C*H*). ^{13}C NMR (DMSO-d_6 + $(PhNH)_2$, 125 MHz): δ = 24.2 (1C), 24.3 (1C), 25.0. (1C), 25.1 (1C), 28.6 (3C), 32.3 (1C), 53.2 (1C), 64.6 (1C), 65.2 (1C), 78.7 (1C), 155.9 (1C), 160.9 (1C), 165.8 (1C), 172.0 (1C), 172.7 (1C). HRMS: (ESI) *m/z*: $[M + H]^+$ calcd. For $C_{17}H_{27}N_5O_5S$ 413.1733, found 413.1720. Anal. Calcd for $C_{17}H_{26}N_5O_5S$ C, 49.50; H, 6.35; N, 16.98; S, 7.77; Found: C, 49.38; H, 6.43; N, 16.85; S, 7.69.

CRediT authorship contribution statement

Yafea Mohsen: Writing – original draft, Investigation, Conceptualization. **Balázs Bognár:** Methodology, Formal analysis. **József Jekő:** Validation, Data curation. **Attila Bényei:** Validation, Data curation. **Tamás Kálai:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tamas Kalai reports financial support was provided by National Research Development and Innovation Office. Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2026.103779.

Data availability

Data will be made available on request.

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