Supplemental Information for
Selenium-Encoded Isotopic Signature Targeted Profiling

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I. Supplemental Figures

Figure S1 Schematic workflow for testing the detection power of SESTAR in a benchmark system. (a) Synthetic selenopeptides were added into water for LC-MS/MS analysis. Excellent selenium-encoded isotopic envelopes could be viewed in the full scan mass spectra and the peptide identities were validated by MS/MS spectra. (b) Synthetic selenopeptides were spiked in a series of dilution (500, 100, 50, 10 and 5 fmols) into 2.5µg of HeLa cell lysates for LC-MS/MS analysis. Data were analyzed by SESTAR and the corresponding MS/MS spectra of the synthetic selenopeptides were manually detected, which allows the comparison of the sensitivity of SESTAR and that of fragmentation by mass spec.
Figure S2 Recovery of native selenopeptides from Human Proteome Map datasets by SESTAR at different combination of score cutoffs. At the cutoff of $S_S \leq 10$, $S_D \leq 1/6$, 59 of the 67 native selenopeptides (88%) were detected by SESTAR and at a slightly loosened cutoff of $S_S \leq 10.25$, $S_D \leq 1/6$, the recovery improved to 62 out of 67 (92%). The five missed selenopeptides had only one selenium-encoded isotopic envelope detected by SESTAR which failed to meet the cutoff of a minimum of two envelopes per peptide.
Figure S3 The averaged transcription level for selenoproteins from different tissues and cell types. The selenoproteins are categorized into three groups: (1) identified by the regular database search; (2) additional detections by SESTAR only; and 3) not detected by either method. Selenoproteins detected by the database search or by SESTAR tend to have higher transcription level than those of not detected. Secreted proteins were excluded in the calculation because they were secreted after maturation so that no correlation would be expected between the transcription and protein level.
Figure S4. Representative MS/MS fragmentation spectra of native selenopeptides from SPS2 and MSRB1. These two selenopeptides were additionally detected by SESTAR-directed targeted analysis from the chemically enriched selenoproteome samples.
Figure S5. SESTAR-directed targeted profiling improved detection of lysine-containing peptides modified by a selenium-containing NSH-ester probe (a)

The synthetic procedure of the Se-NHS ester probe (top) and the schematic view of its reaction with amino side chain of lysine (bottom). (b) SESTAR-directed targeted profiling improved by two fold the detection of lysine-containing peptides from HeLa cell proteomes chemically labeled by a Se-NHS ester probe.
Figure S6. Application of SESTAR to enrich selenium-encoded MS/MS spectra. (a)
Schematic workflow of the application of SESTAR in enriching selenium-encoded MS/MS spectra; (b) Enrichment of selenium-encoded MS/MS spectra by SESTAR significantly reduced the regular database search space and time without losing power to detect native selenopeptides from proteome samples; AU#1: Adult_Urinarybladder_bRP_Elite_71; AU#2: Adult_Urinarybladder_Gel_Elite_70; AT#1: Adult_Testis_bRP_Elite_68; AT#2: Adult_Testis_Gel_Elite_69; (c) Enrichment of selenium-encoded MS/MS spectra by SESTAR not only reduced the regular database search space and time, but also improved the detection of selenium-containing peptides from chemically labeled HeLa proteomes by the Se-NHS ester probe.
Figure S7 Application of SESTAR in targeted analysis of selenoproteome covalently modified by the RSL3-alkyne probe (a) The schematic work flow of profiling the targets of (1S, 3R)-RSL3 with site-specific precision by SESTAR-aided TOP-ABPP. (b) Monitoring of the proteome reactivity of the RSL3-alkyne probe by in-gel fluorescence. Time-dependent labeling of the RSL3-alkyne probe was observed which can be competed by the native RSL3 compound.
Figure S8 MS/MS spectra for the selenopeptides adducted with the RSL3-alkyne probe. For each adducted selenopeptide, the peptide sequence, calculated mass and observed precursor m/z of the “full” adduct are listed. The ion peak corresponding to the “broken” adduct at the ester bond (with about -280.19Da) was indicated with a red arrow. The b/y ions corresponding to the full adduct were indicated as regular b and y. The b/y ions corresponding to the broken adduct were indicated as boxed b and y. The adducted selenocysteine site was labeled with a “U” colored in red.
Figure S9. SESTAR helps to detect other forms of the selenopeptide from SELH. (a) An envelope with strong selenium pattern was detected by SESTAR and the corresponding ms2 spectrum was matched to a modified form of selenopeptide from SELH in adult Bcell, which an intramolecular SbSe bond formed and a deamidation from N to D (b) The same selenopeptide with only the intramolecular S-Se bond but no deamidation was also observed.
II. Supplemental Tables

**Table S1.** Sample information of datasets downloaded from the Human Proteome Map project.

**Table S2.** Overlapping detections of selenopeptides by SESTAR and database search. For selenopeptides detected in multiple raw file of one tissue or cell type, only the one with the best score is shown. Selenopeptides detected by database search only are listed without SESTAR scores.

**Table S3.** Additional selenopeptides detected by SESTAR from different tissues and primary cells. They are broken into three sub-categories according to reliability, which are indicated by different colors.

**Table S4.** List of selenocysteine and cysteine sites covalently modified by the RSL3 probe as identified by SESTAR-aided chemical proteomic analysis.
III. Experimental Methods

Safety Statement

No unexpected or unusually high safety hazards were encountered.

SESTAR Software Algorithm

SESTAR employs full scan spectra in ms1 format as input. The first step is to group peaks into envelopes (Figure 1c). It takes every full scan spectrum as an envelope detection unit and consider all peaks for evaluation. When charge states are unknown, we iterate from 6+ to 1+ to calculate the corresponding mass. After certain peaks have been grouped to one envelope at a specific charge state, they are removed from the peak list and will no longer be considered at lower charge state. For envelope grouping, two peaks with the difference of one isotopic mass unit (1.0033548 Da) are considered as being isotopically related. Given the m/z value and charge state of a certain envelope, its monoisotopic mass is calculated and then used to predict its elemental composition, including or excluding a selenium atom explicitly, by the “averagine” function as implemented previously for quantification of chemproteomic data by activity based proteome profiling (ABPP) studies\textsuperscript{1}. By convoluting the component elements’ isotopic distributions, the isotopic pattern of the proteomic peptide or selenium-containing peptide is simulated.

In the second step, SESTAR decides whether an observed envelope belongs to a selenium-containing envelope or a proteogenic one (Figure 1d). Each envelope is encoded into a vector of relative peak intensities and the function of cosine similarity is employed to measure the similarity of the observed envelope ($E_o$) to either a
simulated selenium-containing \((E_{Se})\) or proteogenic envelope \((E_p)\). The program calculates two scores to make the decision:

1. **Similarity Score** \((S_S)\), which is the cosine similarity between the observed envelope and the simulated selenium-containing envelope.

\[
S_S = \cos^{-1} \frac{E_{Se} \cdot E_o}{|E_{Se}| |E_o|}
\]

2. **Discrimination Score** \((S_D)\), which is the ratio of two respective similarity scores, illustrating to what extent the observed peptide is likely to be selenium-containing rather than proteogenic.

\[
S_D = \frac{\cos^{-1} \frac{E_o \cdot E_{Se}}{|E_o||E_{Se}|}}{\cos^{-1} \frac{E_o \cdot E_p}{|E_o||E_p|}}
\]

For both \(S_S\) and \(S_D\), the smaller they are, the more the peptide is likely to contain a selenium. As the observed envelope is always part of the full envelope, the program slides the observed envelope on the simulated envelope and choose the one with best score as the best alignment.

Previous studies have shown true positive detections are often detected in multiple events\(^2\). To increase the confidence of any assignment, we required it to have at least two associated selenium-encoded isotopic patterns that are detected by SESTAR with qualified scores.

**SESTAR software availability**

The source code of SESTAR was available for download at GitHub (https://github.com/JinjunGao/SESTAR).
Dilutions of Synthetic Selenopeptides in Whole Cell Lysate

Two selenopeptides from SELW (VVYCGAUGYK) and SELT (FQICVSUGYR) were synthesized by GL Biochem (Shanghai) Ltd. with a purity of 98%. HeLa cells were obtained from ATCC and maintained in DMEM supplemented with 10% (vol/vol) FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere at 37 °C with 5% CO₂. Cells were harvested by scraping and centrifugation at 1,400 g, then washed twice in phosphate buffered saline (PBS) Buffer and frozen. Frozen cell pellets were resuspended in PBS Buffer and lysed by sonication. The extracted proteins were quantified with the BCA protein assay kit (Pierce), and normalized to 2 mg/ml. The synthetic selenopeptides were spiked into 2.5 µg Hela lysate with a series of dilutions (500, 100, 50, 10 and 5 fmols). Proteins were then followed by being treated with dithiothreitol (DTT) (Sigma-Aldrich), iodoacetamide (Sigma-Aldrich) and digested by trypsin on-column in 50 mM ammonium bicarbonate (Sigma-Aldrich) buffer and then concentrated by vacuum.

Mass-spec analysis was performed on a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled with Dionex UltiMate3,000 HPLC. Under the positive-ion mode, full-scan mass spectra were acquired over the m/z range from 350 to 1,800 using the Orbitrap mass analyzer with a mass resolution of 70,000. MS/MS fragmentation is performed in a data-dependent mode, of which TOP 20 most intense ions are selected for MS2 analysis with a resolution of 17,500 in HCD collision mode. Other important parameters: isolation window, 2.0 m/z units; default charge, 2+; normalized collision energy, 28%; maximum IT, 50 ms; dynamic exclusion,
Estimate of False Positive Rate by *Arabidopsis thaliana* Proteome

*Arabidopsis thaliana* ecotype Columbia (Col-0) was obtained from the Arabidopsis Biological Resource Center (ABRC, https://abrc.osu.edu/). Whole sprouts on the 11th day after germination were used for proteome preparation. *A. thaliana* sprouts were mechanically homogenized by Dounce homogenizer and subsequently sonicated in 6M urea (Sigma-Aldrich) in PBS buffer. The following quantification and trypsin digestion procedures were performed as described above. Then the vacuum dried peptides were further fractionated to 26 fractions by the Fast-seq protocol\(^3\) to reduce the sample complexity. Mass-spec analysis was performed as described above.

All ions (selenium containing isotopic envelopes) detected in *A. thaliana* proteome were considered as false positives ($FP_{ions}$). Total negatives ($N_{peptides}$) were defined as the total number of peptides in the digested sample (i.e., the number of peptides in *A. thaliana* whole proteome). For this calculation we reasoned that there are about 15,400 reviewed proteins in Uniprot database and that we would expect a total of 10 tryptic peptides per protein, giving 154,000 total peptides. The FPR is calculated as:

$$\text{False positive rate (FPR)} = \frac{FP_{ions}}{N_{peptides}}$$

It should be noticed that the calculated FPR is an over estimation, because: (1) we report ions/peptides instead of peptides/peptides, in this condition multiple ions
may correspond to one peptide due to variety of reasons (e.g., miss-cleavage and post translational modifications); and (2) we cannot formally exclude the possibility of other forms of selenium-containing proteins (e.g., a post-translational modification with selenium or potential bacteria contaminations). So the actual FPR is expected to be better than reported.

Whole Proteome Analysis of Mouse Liver

Male mice (C57BL/6j, Charles River, Beijing, China) were maintained in a temperature-controlled barrier facility with a 12 h light/dark cycle and were given free access to food and water in the Center for Experimental Animals at Peking University, Beijing, China (an AAALAC-accredited experimental animal facility) until liver samples were harvested. The animal procedures were performed in accordance with protocols approved by the Committee for Animal Research of Peking University, China, and conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985).

The mouse liver was mechanically lysed by Dounce homogenizer and subsequently sonicated in 6 M urea (Sigma-Aldrich) in PBS buffer. The quantification and trypsin digestion procedure were performed as described above. Vacuum dried peptides were further fractionated to six fractions by 2 dimensional orthogonal pH reverse phase chromatography (2D sRP-RP) protocol. Mass-spect analysis was performed on a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled with Dionex UltiMate3,000 HPLC. In the first round, mass-spec
analysis was performed as described above except that the TOP 7 most intense ions were selected for MS2 analysis and the isolation window was 1.6 m/z units. In the second round, an inclusion list was generated for targeted analysis as described in the next section below.

Database search was performed by ProLuCID against the reverse-concatenated non-redundant FASTA mouse database compiled from Uniprot database (version 2012). To enable explicit search of selenopeptides, each selenocysteine(U) was converted to cysteine (C) and two differential modifications on cysteine (+57.02147 Da for iodoacetamide alkylation and 104.9659 Da for mass difference from iodoacetamide alkylated U to C) were set. The precursor and fragmentation tolerances were 10 ppm and 60 ppm. ProLuCID search results were filtered and assembled by DTASelect2.0 with a defined peptide false positive rate of 1%.

**Generation of Inclusion List and Targeted Fragmentation**

All LC-MS/MS data were analyzed by database search and SESTAR in parallel. Additional selenium-encoded isotopic envelopes detected by SESTAR were collected to generate an inclusion list, which contains, for each targeted envelop, a m/z value, charge and a retention time window (+/- 10 mins). In order to get as many as true positive for targeted analysis, the score cutoff was loosened to $S_S <= 15$ and $S_D <= \frac{1}{4}$. The replicate sample was then subjected to LC-MS/MS analysis by the same procedure with the inclusion list enabled and dynamic exclusion disabled.
Chemically Enriched Selenoproteome from Mouse Liver

The tissue from male C57BL/6J mice were obtained as frozen samples (Jackson Laboratories). Detailed experiment procedure and database search process was performed as described in the unpublished work by Weerapana and colleagues (the draft manuscript is provided as a supporting file for review only). The SESTAR-directed targeted analysis was performed as described above. Since the MS data was collected on a LTQ Orbitrap Discovery in the Weerapana lab which collects data with relative low resolution, especially for the MS/MS data that was collected in linear ion trap, the precursor and fragmentation tolerances were set as 50 ppm and 0.6 Da respectively.

Chemical labeling and proteomics by Se-NHS Ester probe

1 mL of 2 mg/ml Hela cell lysates was labeled in PBS Buffer with 100 µM NHS probe for 1 hour at 25 °C and added urea to give 6 M urea in PBS buffer. The following trypsin digestion procedure was performed as described above. the vacuum dried peptides were further fractionated to 6 fractions by small scale reverse phase (sRP) protocol. Database search was performed as described above in the “Whole Proteome Analysis of Mouse Liver” section, except that +57.02147 Da on C and +279.0374 on Lysine (K) as modifications were set. The SESTAR-directed targeted analysis was performed as described above.

Chemoproteomic Profiling of selenoprotein targets of (1S, 3R)-RSL3
Cell culture

The HT1080 cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Life) supplemented with 5% FBS (defined FBS, Premium, South America) and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin). Cells were grown at 37 °C in a 5% CO2 humidified atmosphere in 15 cm² dish (Corning, Corning, NY, USA) with media changed every day.

MTT assay.

1.0 x 10⁴ HT1080 cells were plated in 96-well plates with 100 µL of pre-warmed medium per well (six technical replicates). After 12 h, cells were treated with (1S, 3R)-RSL3 or (1S, 3R)-RSL3-Alkyne probe at indicated concentrations in serum-free medium (DMEM) at 37 °C for another 24 h. Cells were then incubated with MTS reagent (CellTiter 96® AQueous One Solution Cell Proliferation Assay) in 100 µL cultured medium at 37 °C for 2 hours and the absorption at 490 nm was measured with a microplate reader (Bio-Rad).

Gel-based ABPP

8 x 10⁶ HT1080 cells were seeded in 15 cm dishes to grow overnight and incubated with 1 µM (1S, 3R)-RSL3-Alkyne probe for 15 min, 30 min, 60 min, 180 min, or 360 min in serum-free medium, For the competitor-treated samples, cells were co-treated with (1S, 3R)-RSL3 as competitor for 360 min. The cells were collected, lysed by sonication in ice-cold PBS containing 1% TritonX-100 and centrifuged at 100, 000g for 30 min to remove cell debris. The protein concentrations were determined by BCA protein assay and normalized to 5 mg/mL. The proteomes were
reacted with 1 mM CuSO$_4$, 100 µM TBTA ligand, 100 µM rhodamine-N$_3$, and 1 mM TCEP for 1 h at room temperature. The proteomes were boiled using sample buffer at 90 °C for 5 min, resolved on 10% SDS-PAGE gels and imaged by ChemiDoc XRS+ (Bio-Rad). The gels were then stained by Coomassie brilliant blue (CBB) to demonstrate equal loading.

- **Profiling of the probe-modified Proteins and Sites by TOP-ABPP**

  $8 \times 10^6$ HT1080 cells were seeded in 15 cm dishes to grow overnight and incubated with 1 µM (1S, 3R)-RSL3 alkyne for 360 min in serum-free medium. The cells were collected, lysed by sonication in ice-cold PBS containing 1% TritonX-100 and centrifuged at 100 000g for 30 min to remove cell debris. The protein concentrations were determined by BCA protein assay and normalized to 2 mg/mL. The proteomes were reacted with 1 mM CuSO$_4$, 100 µM TBTA ligand, 100 µM biotin-N$_3$ with an acid-cleavable linker, and 1 mM TCEP for 1 h at room temperature. The precipitated proteins were collected by centrifugation (8,000 g, 5 min), washed with cold methanol three times, and resuspended in 1.2% SDS/PBS. The samples were then diluted to 0.2% SDS/PBS and incubated with 100 µL of streptavidin agarose beads (Thermo Scientific) for 4 h at 29 °C. The beads were washed with 3 x 5 ml of PBS and water sequentially, and suspended in 500 µL of 6 M urea/PBS. After reduction with 10mM dithiothreitol (DTT) at 37 °C for 30min and alkylation with 20mM iodoacetamide (IAA) at 35 °C for 30min in dark, the beads were resuspended in 200 µL of 2 M urea, 1 mM CaCl$_2$, and 2 µg trypsin (Promega) in PBS at 37 °C with agitation overnight. On the next morning, trypsin sample was collected, concentrated, separated by the sRP
The samples were subjected to regular shotgun proteomic analysis to obtain the list of proteins targets labeled by the RSL3-alkyne probe.

To identify the actual residue site covalently modified by the probe, the beads were collected by centrifugation and washed with 3 x 1 mL PBS, 3 x 1 mL \( \text{H}_2\text{O} \), followed by acid cleavage. The sample was divided into two equal portions. The first aliquot was analyzed by standard DDA as described above and an inclusion list was generated by SESTAR to direct the paralleled reaction monitoring (PRM) analysis on the second aliquot. The list of probe-adducted selenopeptides was obtained by the ProLuCID search with 2 differential modifications on C (718.256 Da for the probe adduct modification on SeCys, and 57.02146 Da for iodoacetamide alkylation). The list of probe-adducted cysteine-containing peptides was obtained by a separate ProLuCID search with 2 differential modifications on C (670.3115 Da for the probe adduct modification on Cys and 57.02146 Da for iodoacetamide alkylation).

**Match of SESTAR-detected envelopes to known selenopeptides**

For each selenopeptide from trypsin digestion of selenoproteins *in silico*, no more than one missed cleavage event was allowed and only those with one selenocysteine were chosen, resulting in a list of 86 unique selenopeptide sequences with corresponding masses. When the envelope detected by SESTAR has a mass difference less than 10 ppm to any mass in the compiled list of known selenopeptides, it was considered as a potential match.
Human Proteome Map Data Analysis

1676 raw files were downloaded from the Human Proteome Map project via the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) with the dataset identifier PXD000561 (Table S1). The data were generated by shotgun proteomic analysis of 17 adult tissues and 6 purified primary haematopoietic cells. Transcriptome data were downloaded from the Human Protein Atlas project (https://www.proteinatlas.org/). The proteome data were searched by ProLuCID first to detect selenopeptide PSMs and then analyzed by SESTAR to screen additional selenopeptides. Database search process was performed as described above in the “Whole Proteome Analysis of Mouse Liver” section.

For those potential matches to known selenopeptides as detected by SESTAR only, the corresponding m/z values were calculated according to their charge states and traced back in the corresponding ms2 file to find any associated MS/MS spectra. If an MS/MS spectrum exists, it was analyzed by manual assignment. Otherwise, it was checked with the corresponding selenopeptide validated by MS/MS spectra from other data files to determine whether it was eluted at a similar retention time (+/- 5 mins). If no co-eluting reference peptide and no MS/MS spectra was found, the cutoffs S_a <= 10 and S_o <= 1/6 was used as a filter to obtain selenium-encode isotopic envelops with high confidence. All the results above were summarized in Table S2 and Table S3 as a tissue-specific selenoproteome atlas.

For DDA-based search by ProLuCID, one “time” means one unique peptide match based on MS/MS spectra from the specific tissue sample. For SESTAR
analysis, one “time” means one unique peptide match based on all relevant cutoffs including good SESTAR scores (good selenium-containing pattern) and correct precursor mass to known selenopetides.

**Synthesis of the Se-NHS Ester Probe**

The probe synthetic procedure is illustrated in Figure S5. To a solution of 1,4-dioxane/H₂O (1:1, 40 mL) were dissolved L-selenomethionine (1.96 g, 10 mmol) and NaHCO₃ (2.52 g, 3.0 eq), followed by addition of a solution of Boc₂O (3.27 g, 1.5 eq) in dioxane (20 mL) over 30 min. The solution was stirred at room temperature for 24 h. Then the organic solvent was removed and solution was acidified to pH 2-3 using 1 M HCl. The aqueous solution was extracted with ethyl acetate (40 mL) thrice. The combined organic layers were dried over Na₂SO₄. The solution was concentrated and compound 2 was obtained as a yellow solid without further purification (2.70 g, 91% yield).

To an ice-cooled solution of compound 2 (1.49 g, 5 mmol) and N-hydroxysuccinimide (0.64 g, 1.1 eq) in dry CH₂Cl₂ (50 mL), was added DCC (1.24 g, 1.2 eq) slowly and stirred for 30 min at 0 °C. Then the reaction mixture was warmed to room temperature and stirred for 12 h. The reaction mixture was filtered and the filtrate was diluted with 50 mL of CH₂Cl₂, washed sequentially with diluted HCl, saturated NaHCO₃ solution and brine. The organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to give crude product. The crude product was further purified by silica gel column chromatography (dichloromethane/ethyl acetate, 20:1) to yield 3 (1.10 g, 56%) as a white solid.
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.20-5.05 (m, J = 7.4 Hz, 1H), 4.90-4.78 (d, J = 4.8 Hz, 1H), 2.85 (s, 4H), 2.76 – 2.54 (m, 2H), 2.40 – 2.26 (m, 1H), 2.26 – 2.13 (m, 1H), 2.03 (s, 3H), 1.46 (s, 9H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 168.7, 168.1, 154.8, 80.7, 52.1, 33.4, 28.3, 25.6, 19.7, 4.3.
HRMS (ESI, m/z) calcd for C_{14}H_{22}N_{2}NaO_{6}Se (M+Na+): 417.0541; found, 417.0535
and calcd for C_{14}H_{22}KN_{2}O_{6}Se (M+K+): 433.0280; found, 433.0274.

Synthesis of (1S, 3R)-RSL3-Alkyne Probe

(1S, 3R)-RSL3-Alkyne probe was prepared using the synthetic route in reported paper^{10}.

{^1}H NMR (500 MHz, DMF) δ 11.05 (s, 1H), 7.89 (d, J = 7.0 Hz, 2H), 7.67 (d, J = 7.0 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 7.1 Hz, 1H), 7.15 – 6.93 (m, 3H), 6.20 (s, 1H), 5.57 (s, 1H), 4.98 (s, 2H), 4.85 (d, J = 13.7 Hz, 1H), 4.52 (d, J = 13.7 Hz, 1H), 3.72 (d, J = 15.2 Hz, 1H), 3.57 (s, 4H).
$^{13}$C NMR (126 MHz, DMF) δ 172.2, 168.5, 165.2, 150.2, 137.5, 134.1, 129.7, 128.1, 126.9, 126.4, 121.8, 119.3, 118.3, 111.6, 104.5, 78.6, 77.0, 57.3, 57.1, 52.6, 52.4, 43.5, 23.8.
HRMS (ES+) exact mass calculated for [M+H]+ (C_{25}H_{22}ClN_{2}O_{5}) requires m/z 456.1212, found m/z 456.1212.
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