SUPPORTING INFORMATION

Small-Molecule Inhibitors of the CD40–CD40L Costimulatory Protein-Protein Interaction

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Scheme S1: Synthesis of compound **5**.

Scheme S2: Synthesis of compound **7**.

Scheme S3: Synthesis of compound **11**.

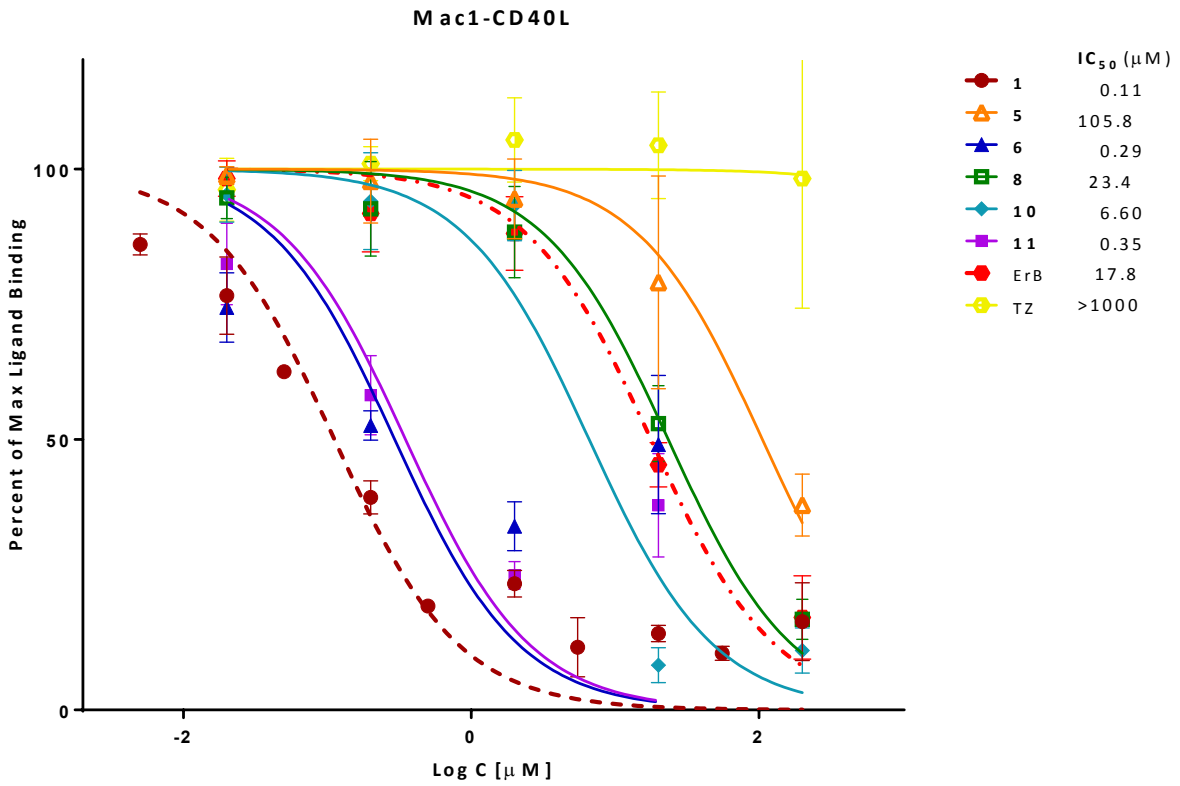


Figure S1. Concentration-dependent inhibition of the human Mac-1–CD40L interaction by selected compounds quantified using a cell-free ELISA-type assay and fitted with standard binding curves. In addition to the organic dye **1** included for comparison, tartrazine (TZ) and the promiscuous PPI inhibitor erythrosine B (ErB) were also included as negative and positive controls, respectively. Data are average ± SD (normalized to percent binding) for n = 3 independent experiments with duplicates for each condition; corresponding IC_{50} values are shown on the right.

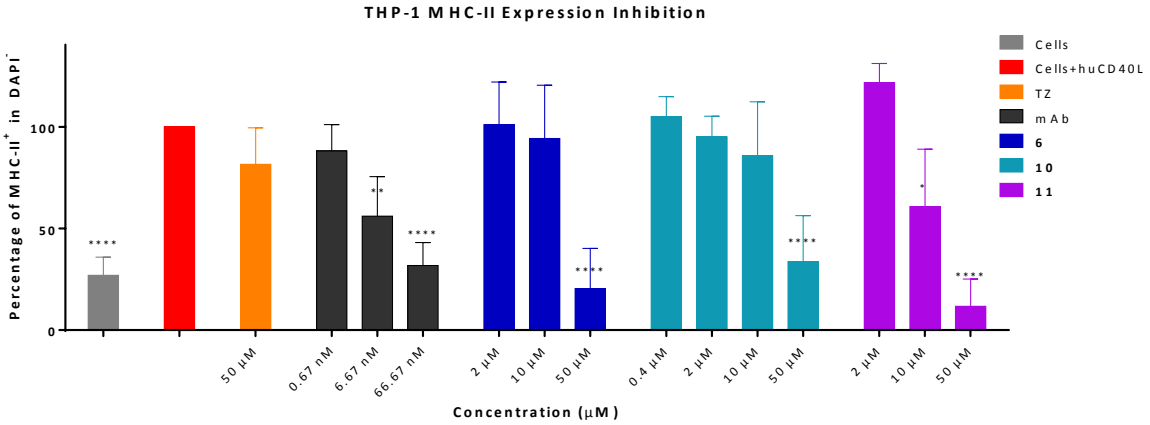


Figure S2. Concentration-dependent inhibition of CD40L-induced (0.5 µg/mL MegaCD40L; Enzo Life Sciences) MHC-II upregulation in THP-1 cells by the present novel compounds (**6**, **10**, and **11**) with tartrazine (TZ) and an anti-CD40L antibody (mAb) included as negative and positive controls, respectively. Data are average ± SD in live ($DAPI^-$) cells from five independent experiments and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$ vs. cells + huCD40L.

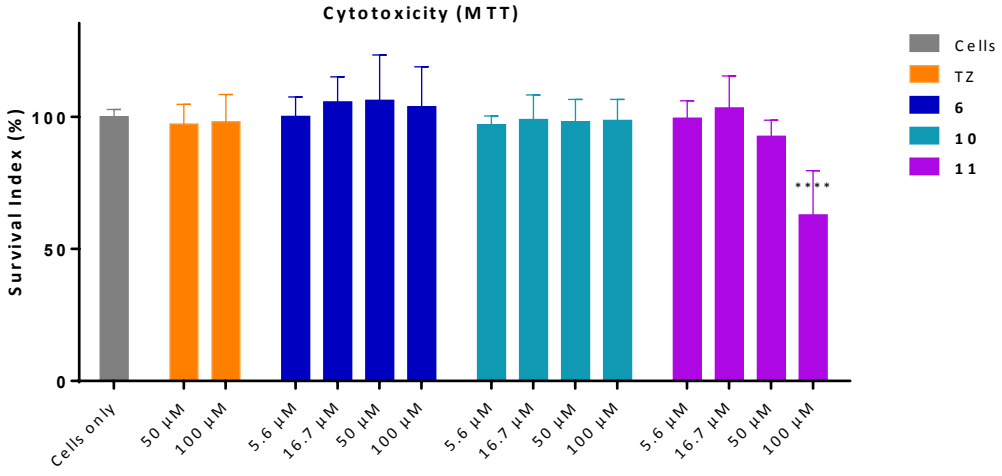


Figure S3. Toxicity assessment of selected new compounds (e.g., **6**, **10**, and **11**) with tartrazine (TZ) as a negative control using a standard MTT cytotoxicity assay in THP-1 cells. Data are average ± SD of three experiments in duplicate and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, $^{****}p < 0.0001$ vs. cells only.

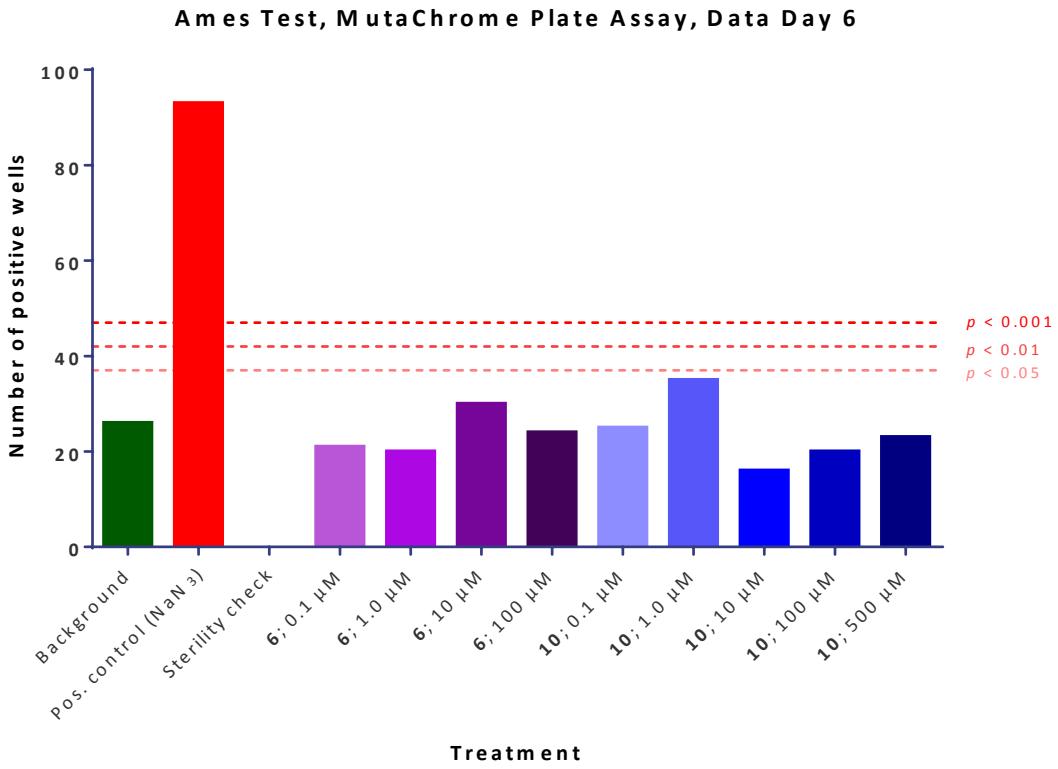


Figure S4. Assessment of mutagenicity (Ames test) for compounds **6** and **10** using a Muta-Chromplate assay. Data are the number of positive cells from 96-well microplates (one plate per condition; reading of day 6), and the horizontal dashed red lines indicate the number of positive wells needed for statistically significant effects at the indicated level.

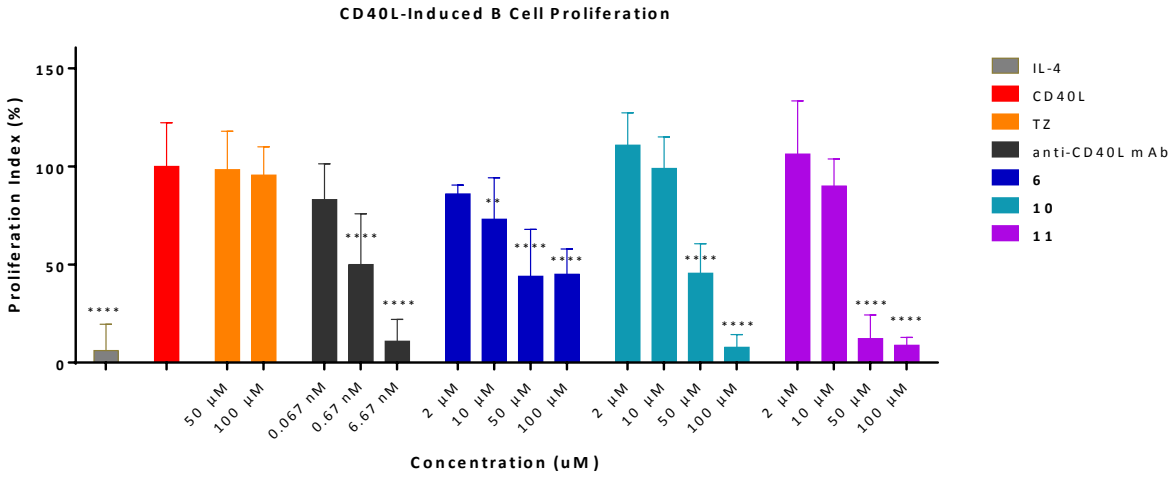


Figure S5. Concentration-dependent inhibition of CD40L-induced (0.1 µg/mL *Mega*CD40L, Enzo Life Sciences) human B cell proliferation by compounds **6**, **10**, and **11** with tartrazine (TZ) and an anti-CD40L monoclonal antibody (mAb) included as negative and positive controls, respectively. Data are average ± SD for $n = 3$ independent experiments with triplicates for each condition using a standard BrdU assay (Roche). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, $^{**}p < 0.01$, $^{****}p < 0.0001$ vs. CD40L.

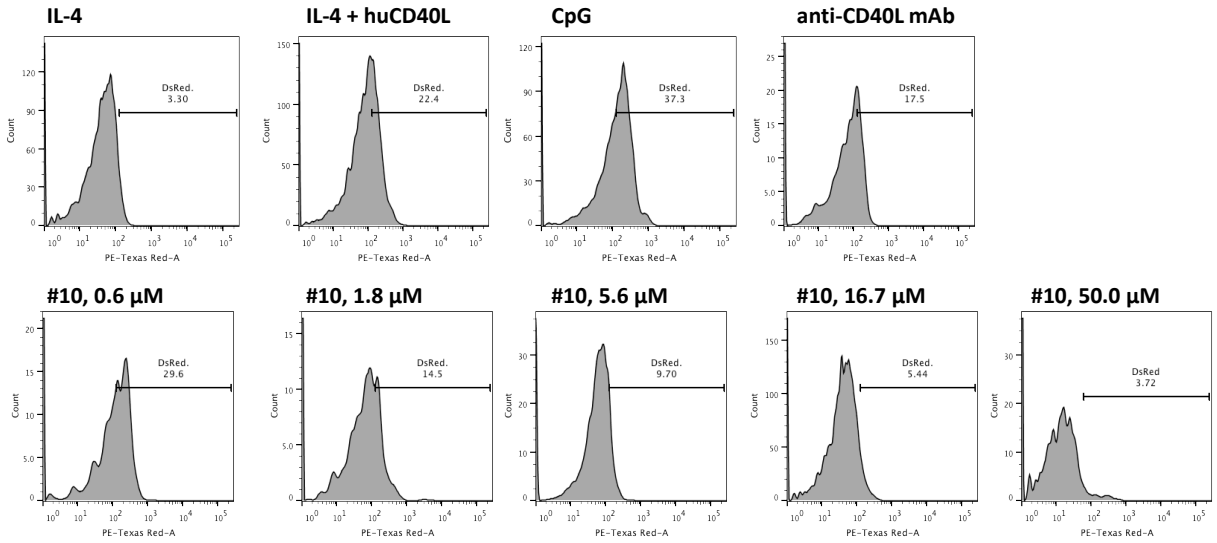


Figure S6. Representative flow cytometry histograms illustrating the concentration-dependent inhibition of CD40L-induced human B-cell function (AID activation quantified via a fluorescent marker detected by flow cytometry) by compound **10** with corresponding controls as indicated corresponding to the average data shown in Figure 6.

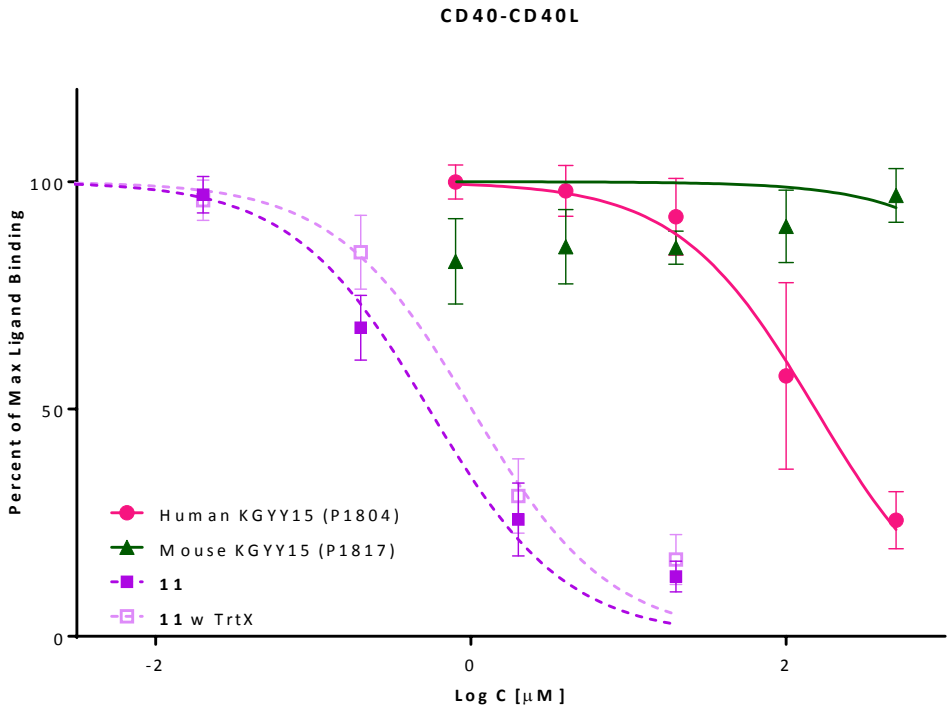
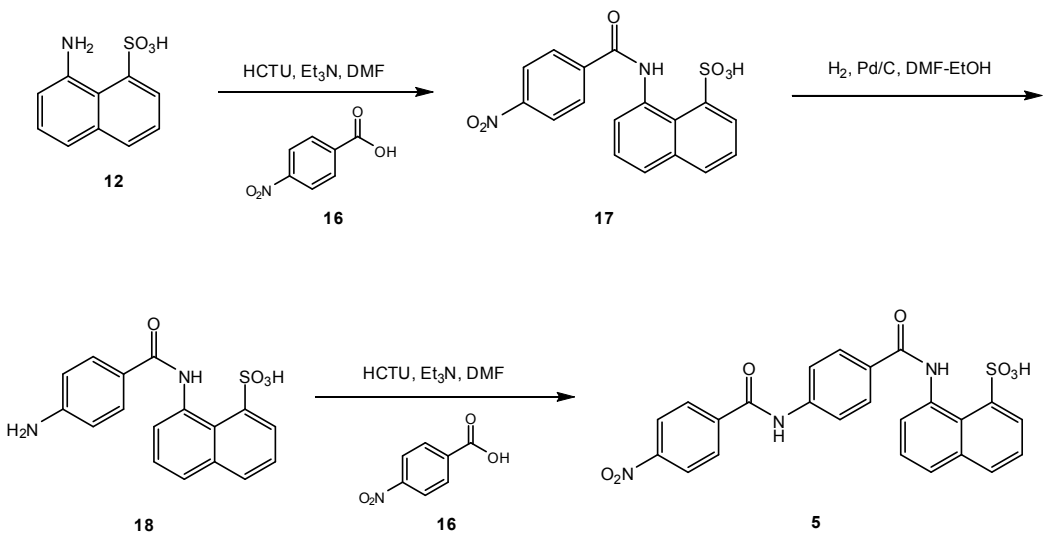
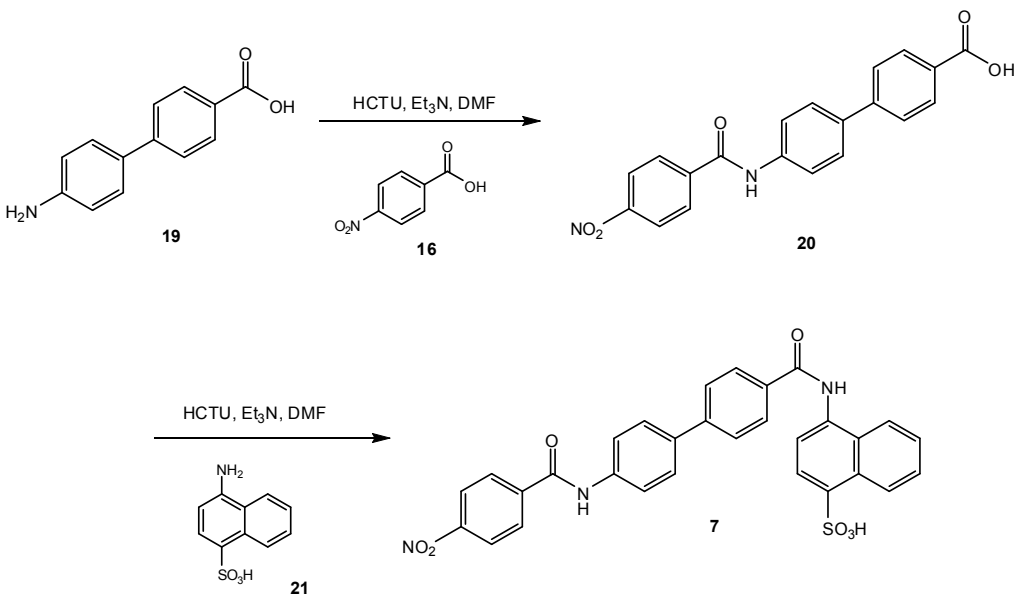


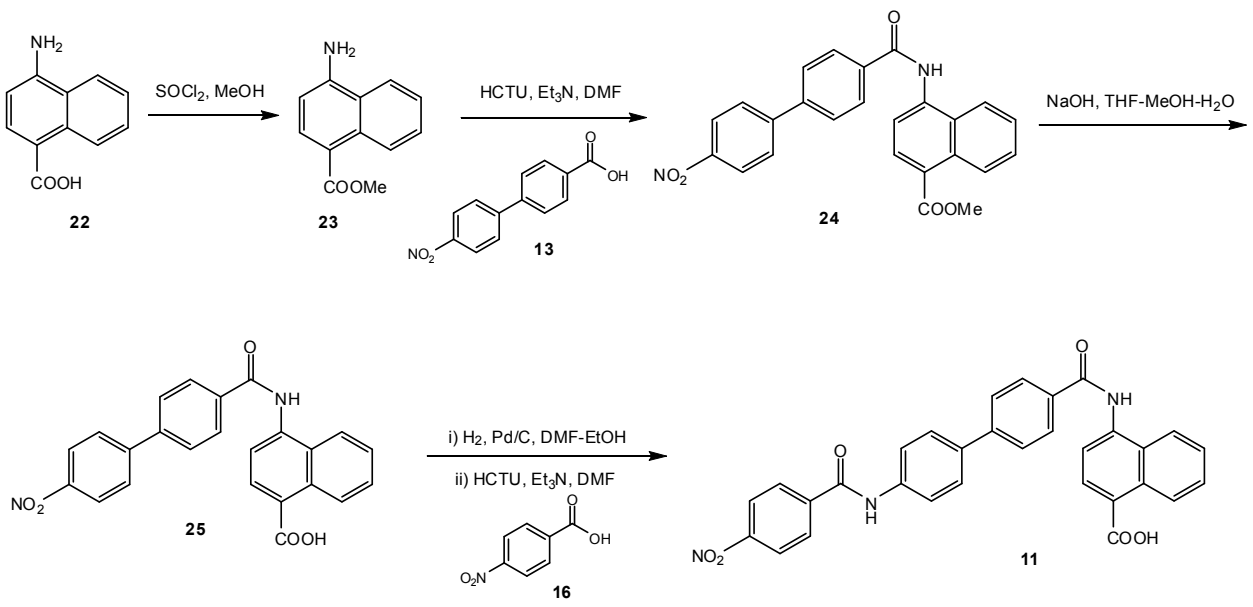
Figure S7. Blocking activity of $KGYY_{15}$ 15-mer CD40 peptide inhibitors [19] in the present human CD40–CD40L binding assay. Mouse $KGYY_{15}$ (VLQWAKKGYYTMKSN) and human $KGYY_{15}$ (VLQWA**E**KGYYTM**SN**N) were tested in the same ELISA assay (Figure 4) as all other inhibitors here giving IC_{50}s of >5000 and 154 μM, respectively. For comparison, the inhibitory activity of **11** is also included as retested with or without added Triton-X 100 (0.01%), a non-ionic detergent used to check that the inhibitory effect is not due to aggregation (IC_{50}s of 1.01 and 0.54 μM, respectively).



Scheme S1. Synthesis of compound **5**.



Scheme S2. Synthesis of compound **7**.



Scheme S3. Synthesis of compound **11**.