New perspectives on aryl azide noncanonical amino acid use in yeast.

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ELECTRONIC SUPPLEMENTARY INFORMATION
Routine HPLC was performed on an Agilent 1100 series with an Alltech 250 mm, I.D. 4.6 mm, C8 5µ column. Purification was performed using a Purospher® STAR RP-18e 5µm HitHunter® 50-21.2mm column. The 4-azido-L-phenylalanine, 4-aminophenylalanine, pBpa were acquired from BACHEM. The solution of 1-azidobromobenzene bromophenylalanine were acquired from Sigma Aldrich; 4-bromoaniline was acquired from Alfa Aesar. Yeast was purchased commercially (Fleischmann’s). [It should be noted the same yeast had recently been used, successfully, by >95% of ~75 undergraduates as they performed a chiral reduction of ethylacetocacetate.] IR analysis was performed on a Thermo Fisher Nicolet iS50 FTIR Spectrometer. Photolysis was performed on a Stratagene 2020e transilluminator at 302 nm UV in quartz vials. NMR spectra were recorded on a 200 MHz Varian/Agilent instrument.

HPLC analysis details:
(solvent A= 0.85% Phosphoric acid; Solvent B = methanol):
Method A. 1. 80:20 A:B 1 min. 2. Ramping to 20:80 A:B 10 min.
Method B (fast elution): 1. 60:40 A:B ramping to 5:95 A:B over 8 min.

General Azide synthesis:
3-(4-Aminophenyl)propionic acid (0.16 g, 1 mmol) was added to CH₂Cl₂ (650 µL) in a 10 mL conical flask. Et₃N (420 µL) was added where upon the propionic acid dissolved. Next, a solution of CuSO₄ (8 mg, 0.05 mmol; 200 µL DI water) was added to the flask. The TfN₃ was freshly prepared in dichloromethane according to Liu and Tor and added to the reaction followed by ~600 µL of MeOH which brought about homogeneity. The reaction solution was stirred at room temperature for 2 h. The reaction mixture was then poured into saturated aqueous NaHCO₃ (10 mL) and extracted with CH₂Cl₂ (3x10 mL). The combined organic phases were washed with brine (10 mL), dried over MgSO₄, filtered through silica gel and concentrated to a crude oil. ¹H NMR (200 MHz, CDCl₃) δ 2.5 (t, 2H, J = 6.8 Hz), δ 2.89 (t, 2H, 7.9 Hz), δ 6.85 (d, 2H, J = 8.2 Hz), δ 7.08 (d, 2H, J = 8.2 Hz); IR (neat) 2114 cm⁻¹ (strong; N₃-vibration). HPLC analysis (Method A: retention time = 8.425 - 8.433 min).
Supporting Figure S1. Relative amino acid concentrations followed as a function of time. Percent abundance relative to the initial $t = 0$ h- HPLC with UV analysis: grey = azidophenylalanine; yellow = bromophenylalanine; blue = aminophenylalanine.
Supporting Figure S2. HPLC analysis of a yeast culture supplemented with 4-azidophenylalanine (blue trace) or para-benzyolphenylalanine (red Trace) at 2 h. The benzophenone-derived metabolite was not characterized in any way besides retention time; the metabolite peak only appears in yeast cultures supplemented with para-benzyolphenylalanine and is ostensibly of a similar polarity to the AzF metabolite due to the similar retention time. p-bromophenylalanine and aminophenylalanine also produced new, prominent metabolites with unique retention times in the 6.5-7.5 min region (data not shown). HPLC was performed with Method A.
Supporting Figure S3. Simultaneous HPLC comparison of synthesized azides with a yeast culture metabolite (i.e. 2-(4-azidophenyl)ethanol) extracted from (+) AzF cultures. HPLC analysis was performed with Method A.
Supporting Figure S4. Photochemical analysis of a yeast culture metabolite (i.e. 2-(4-azidophenyl)ethanol) and a synthesized azide, 4-azidophenylpropenoic acid. In the blue trace the compounds were combined into one solution prior to photolysis. The red trace is the same solution after 25 min at 302 nm photolysis in a quartz reaction tube. HPLC was performed with Method A.
Supporting Figure S5. Comparison of HPLC chromatograms of a AzF-derived metabolite (ostensibly from 2-(4-aminophenyl)ethanol) from yeast cultures and a synthesized 4-azidophenylethanol. The blue trace is 2-(4-azidophenyl)ethanol independently synthesized from 2-(4-aminophenyl)ethanol. The red trace is from a CH$_2$Cl$_2$ extract of a yeast (+) AzF culture at 5.5 h. HPLC used was Method A.
Supporting Figure S6. ¹H-NMRs of 2-(4-azidophenyl)ethanol extracted from *S. cerevisiae* cultures supplemented with azidophenylalanine (top); and 2-(4-azidophenyl)ethanol independently synthesized from 2-(4-aminophenyl)ethanol (note: ethyl acetate used during extraction is clearly visible as a minor component). The alcohol O-H absorbance observed at 2 ppm undergoes deuterium exchange on the order of hours and disappeared from the synthesized sample as well (data not shown).
Supporting Figure S7. FTIR spectrum of AzF metabolite derived from yeast cultures showing prominent and intact azide vibration ~2100 cm$^{-1}$ (top, blue) compared with a spectrum from independently synthesized 2-(4-azidophenyl)ethanol sample (bottom, red).
Supporting Figure S8. Determination of 4-bromoaniline response factor using HPLC and integration of the 254 nm peak area. The 4-bromoaniline was obtained from a commercial source and used as-is.
Supporting Figure S9. Representative HPLC chromatograms from a low-nanomole determination of 4-bromoaniline’s response factor curve. The traces were linear in this region and represent HPLC analysis of a commercial source of 4-bromoaniline. The absorbance and time axes are offset by 10% each.
Supporting Figure S10. The Blue trace is a from a response factor determination injection of low nanomole amounts of commercial sample of 4-bromoaniline. The Red trace is after 24 h incubation of bromophenylazide at 30 deg in the presence of yeast. The initial incubation quantity added to the yeast was ~50 mmoles of 4-bromophenylazide. The absorbance and time scales are not offset. Analysis was performed with HPLC Method A.
Supporting Figure S11. Reduction of azidophenylalanine by dithiothreitol (DTT). Aminophenylalanine retention time was confirmed using a commercially available sample. The absorbance axis is offset by 10%; the time axis is not offset. In the absence of DTT, the concentration of azidophenylalanine remained unchanged after 24 h at 55°C in water alone (data not shown). The relative molar extinction coefficients are not factored into the chromatograms. HPLC analysis performed using Method A.
Supporting Figure S12. A. Crystal structure of a complex between the middle segment of Hsp90 (M-Hsp90: residues 272–530) and the N-terminal domain of Aha1 (N-Aha1: residues 1–153) deposited at PDB (1USU). The region (59–66) for the incorporation of the non-canonical amino acid within N-Aha1 is highlighted in red. B. Chemical structure of pAzpa and pBzpa. C. Expression of Aha1 in the presence and absence of the non-canonical amino acid pAzpa incorporated at the indicated positions.

Supporting Figure S13. Response factor determination of azidophenylalanine. The response factor was used to quantify [AzF] concentrations in (+) AzF supplemented yeast cultures.