Supporting Information – 3 Figures:

OYE Flavoprotein Reductases Initiate the Condensation of TNT-Derived Intermediates to Secondary Diarylamines and Nitrite

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6 pages and 3 figures

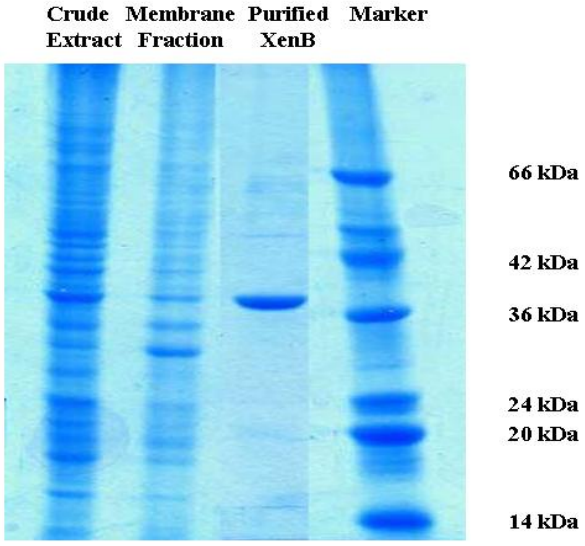


FIGURE S1. SDS-PAGE analysis of the purification of recombinant XenB from *Pseudomonas putida* JLR11. Crude extract from *E. coli* BL21 pLysS harboring the expression plasmid pET28b+ is shown in the first lane. The analysis of the membrane fraction shows that some XenB was co-precipitated during work-up by centrifugation. The expected size of XenB is 37 kDa.

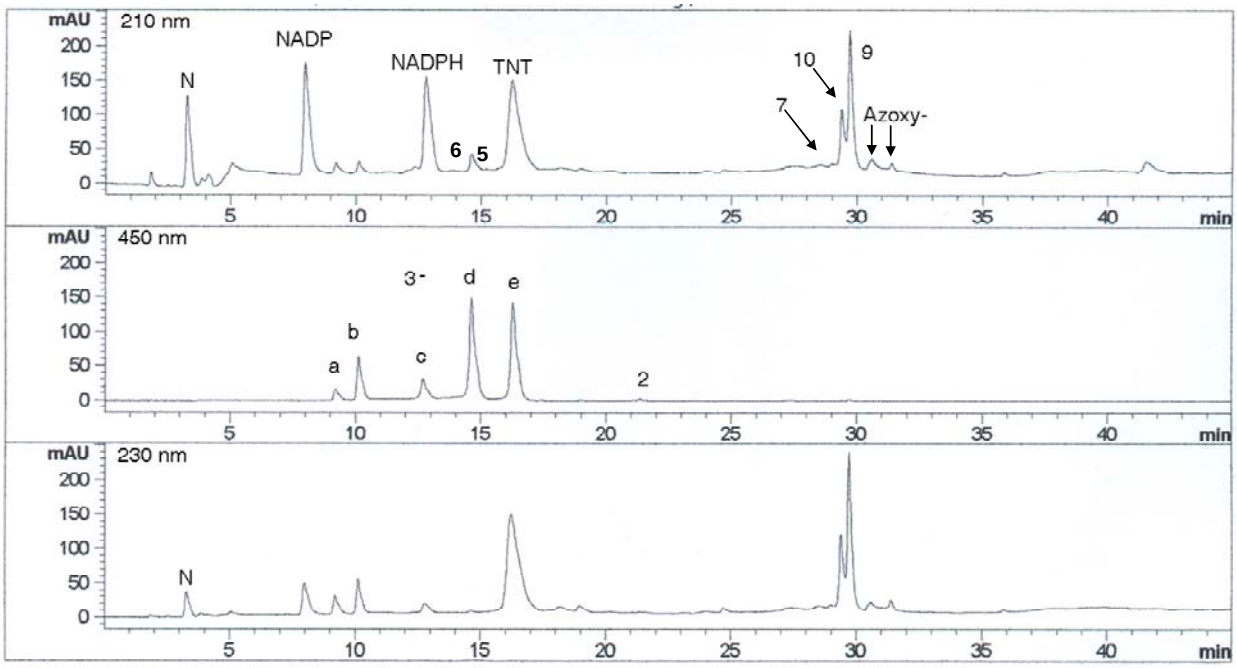
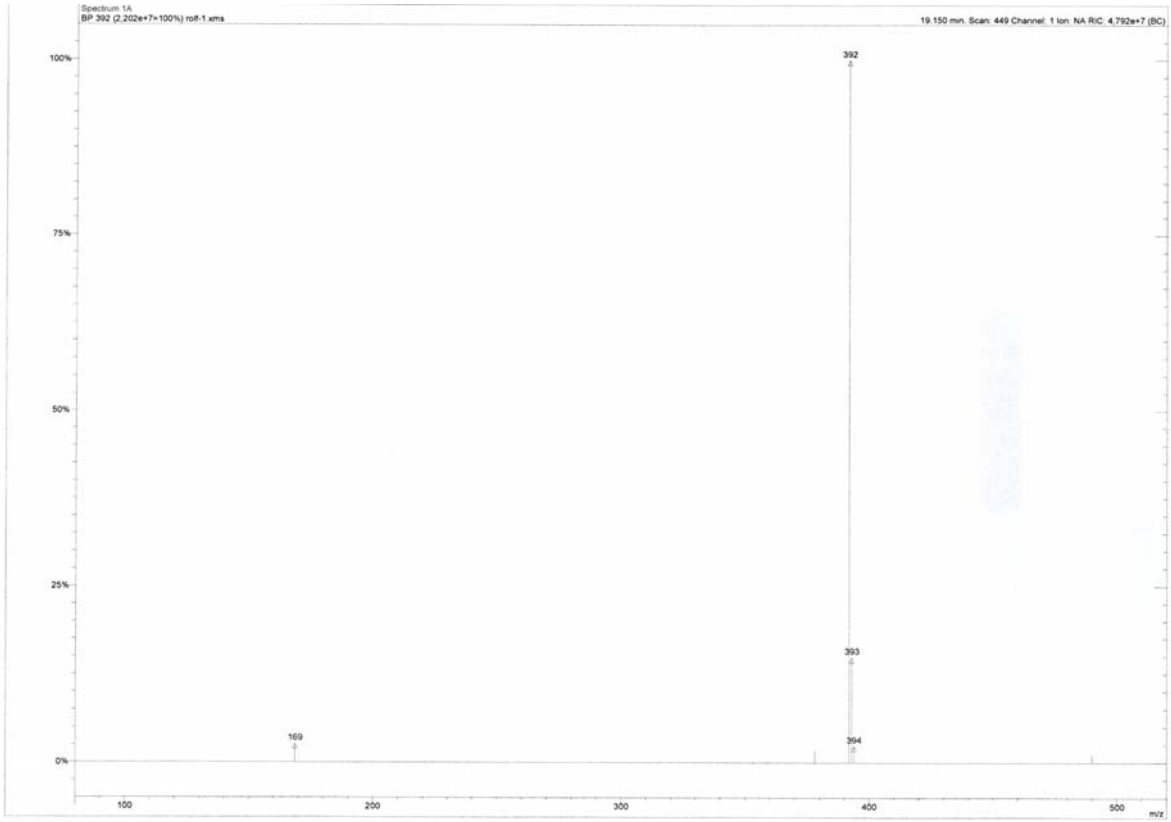
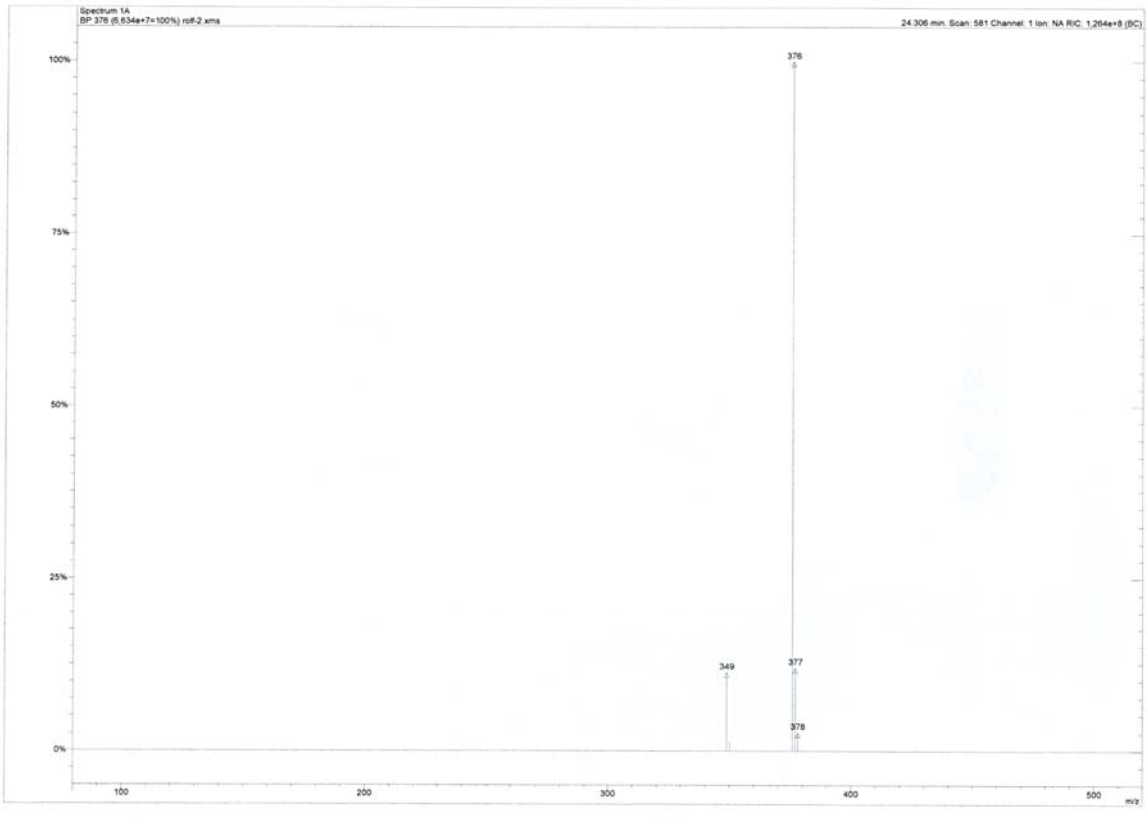


Figure S2. Representative separation by HPLC-DAD of metabolites of 2,4,6-trinitrotoluene (TNT) upon transformation by XenB after 55 min. The position of elution of individual compounds has been marked with the corresponding number; for explanation, refer to Figure 1; N, nitrite, for Azoxy- see the two isomers in the Materials and Methods section, as well as for the isoforms of the Meisenheimer hydride complexes. Note that hydroxylaminodinitrotoluenes (5, 6) do not accumulate due to condensation to compounds 9 and 10; 7 is present only in trace amounts.

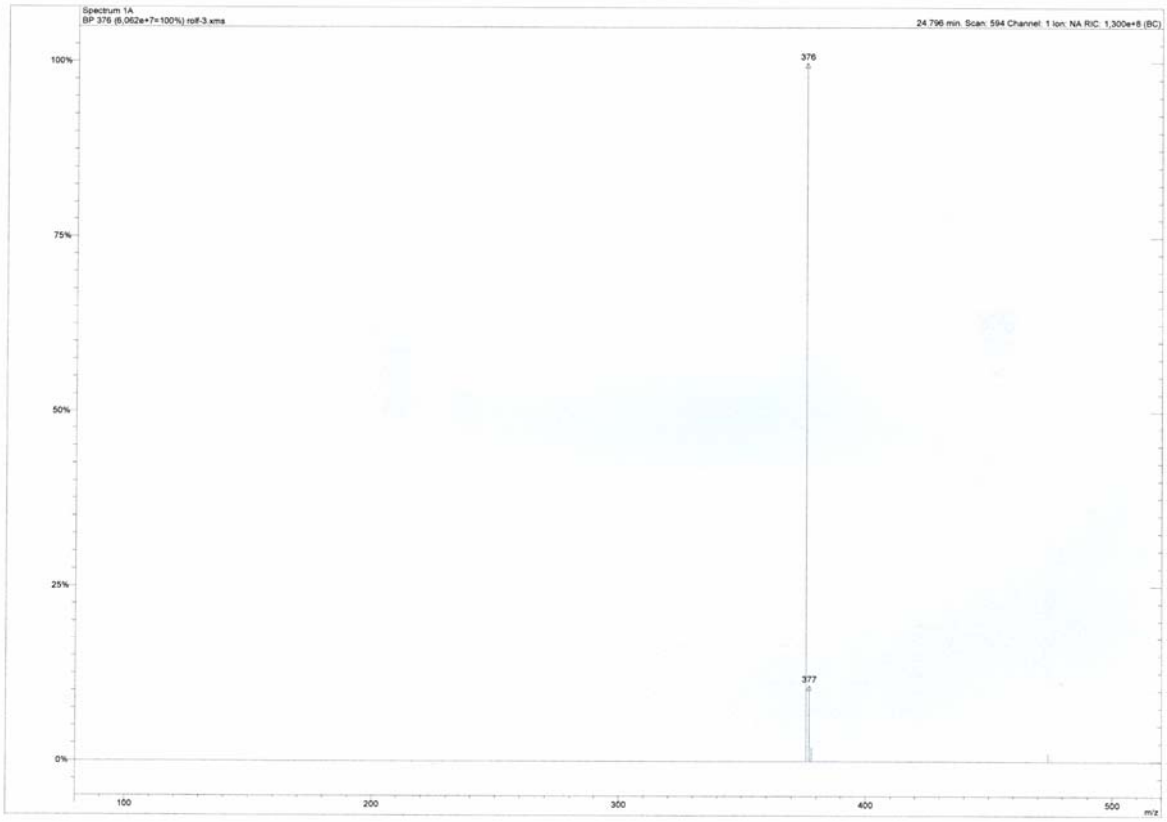
A


Spectrum 1A
BP 392 (2.202e+7=100%) rolf-1.xms
19.150 min. Scan: 449 Channel: 1 Ion: NA RIC: 4.792e+7 (BC)
100%
75%
50%
25%
0%
392
393
394
169
100
200
300
400
500
m/z

B


Spectrum 1A
BP 376 (6.634e+7=100%) rolf-2.xms
24.306 min. Scan: 581 Channel: 1 Ion: NA RIC: 1.264e+8 (BC)
100%
75%
50%
25%
0%
376
377
378
349
100
200
300
400
500
m/z

C


Spectrum 1A
BP 376 (6,062e+7=100%) rolf-3.xms
24.796 min. Scan: 594 Channel: 1 Ion: NA RIC: 1,300e+8 (BC)
100%
75%
50%
25%
0%
376
377
100
200
300
400
500
m/z

D

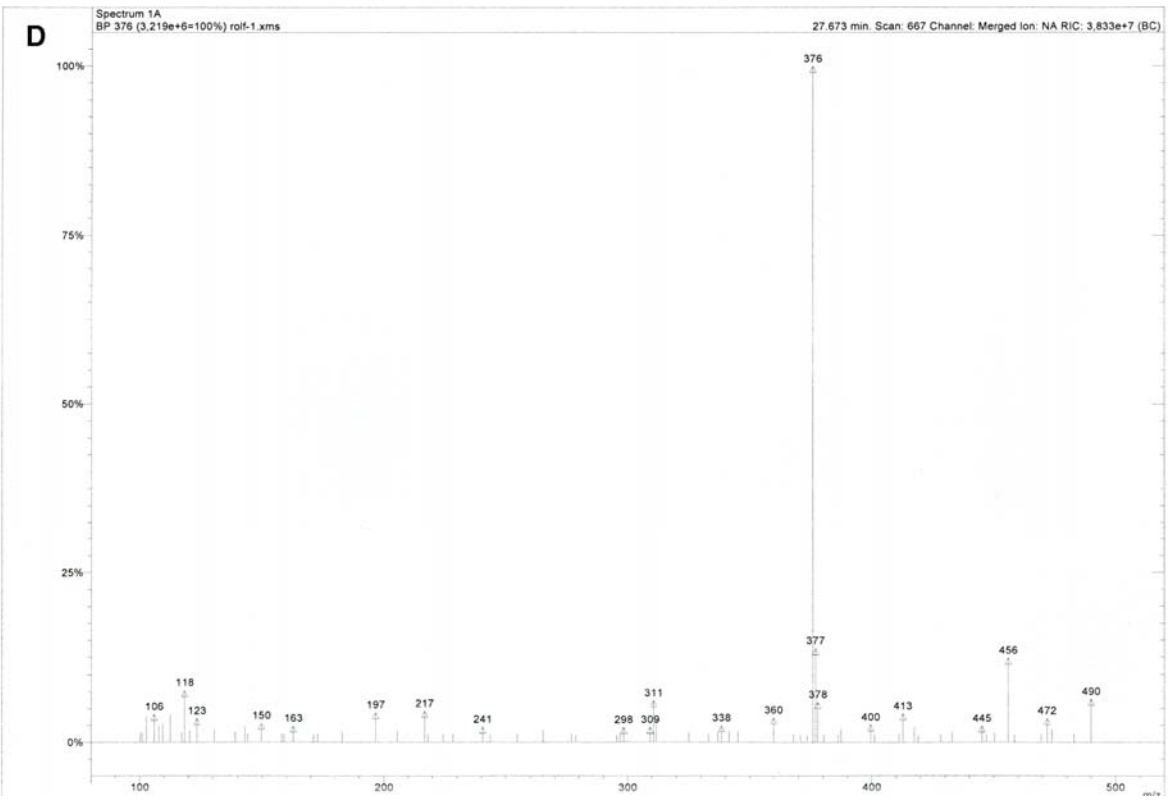

Spectrum 1A
BP 376 (3,219e+6=100%) rolf-1.xms
27.673 min. Scan: 667 Channel: Merged Ion: NA RIC: 3,833e+7 (BC)
100%
75%
50%
25%
0%
376
377
378
106
118
123
150
163
197
217
241
298
309
311
338
360
400
413
445
456
472
490
100
200
300
400
500
m/z

FIGURE S3. LC/MS spectra of chemically synthesized compounds **7** (A), **9** (B), **10** (C), and of the biologically produced compounds **9** and **10** by the XenB enzyme reaction, which reproduce fully identical mass spectra (D, only one of both shown).