SUPPORTING INFORMATION

Squaric acid as a new chemoselective moiety for mass spectrometry-based metabolomics analysis of amines

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1. Supporting schemes

**Scheme S1**: Preparation of chemical probe activated for amine conjugation.

**Scheme S2**: Preparation of simplified chemical probe activated for amine conjugation.
2. Supporting figures

**Figure S1**: Extracted ion chromatograms resulting from reactivity experiments in different condition. Conjugated 1-aminopropane (4a), piperidine (4b), aniline (4c), L-serine (4d), N-acetyl-L-cysteine (4e)

**Figure S2**: Extracted ion chromatograms resulting from stability experiments. 4c and 4d for treatment with Pd(OAc)$_2$, PPh$_3$, dimethylbarbituric acid in THF, 16 h, 25 °C. Intensities were normalized to 100% for each EIC separately.
Figure S3: The first 55 of the total 165 metabolites detected after analysis of fecal samples using the amine-specific chemical probe. Red indicates no detection; Green denotes detected metabolites, which are annotated on the basis of m/z value as an output from the XCMS analysis.

| Conjugates [M+H]⁺ | RT/min | 6M 18M 21F | Annotation |
|-------------------|--------|------------|------------|
| 319.1401          | 5.72   |            | Ammonia    |
| 363.1658          | 5.94   |            | Ethanolamine |
| 373.1869          | 7.00   |            | Pyrrolidine |
| 377.1449          | 7.16/6.60 |          | Glycine    |
| 387.2024          | 7.73   |            | Piperidine |
| 389.1456          | 10.02/10.95 |        | 2-Aminoaacryl acid |
| 391.1608          | 6.79   |            | Beta-Alanine; L-Alanine; Sarcosine; D-Alanine |
| 392.1557          | 5.88   |            | (Aminomethyl)carboxylic acid |
| 401.2190          | 7.79   |            | Cyclhexylamine; 2-Methylpiperidine |
| 403.1973          | 6.39   |            | 5-Aminopentanal |
| 405.1396          | 8.76   |            | 3-Oxosalanine |
| 407.1558          | 9.05/10.44/9.33 |      | L-Serine; D-Serine; Protein serine |
| 417.1764          | 6.70   |            | L-Proline; D-Proline; 4-Amino-2-methylenbutanonic acid |
| 418.2084          | 6.06   |            | 5-Aminopentanamide |
| 419.1820          | 8.08   |            | L-Valine; N-Methyl-a-aminobutyric acid; 5-Aminopentanonic acid |
| 420.1876          | 7.43   |            | 2,4-Diaminobutyric acid; L-2,4-diaminobutyric acid |
| 425.1810          | 8.99   |            | p-Aminidole; 2-Proapionylpyrrole; 4-Hydroxybenzylamine |
| 427.1278          | 6.04   |            | Taurine |
| 428.1778          | 5.12   |            | Melanine |
| 431.1926          | 7.56   |            | 1-Piperidine carboxylic acid; Piperocolic acid; D-Piperocolic acid; |
| 432.2327          | 6.2/7.13 |          | N-Acetylputresine |
| 433.1716          | 9.70   |            | 4-Hydroxyproline; 5-Aminolevulinic acid; L-Glutamic gamma-semialdehyde; |
| 435.2073          | 8.86   |            | L-Isoleucine; L-Alloisoleucine; L-Leucine; L-Norleucine; Aminocaproic acid; |
| 435.1869          | 9.83   |            | L-2-Amino-5-hydroxyxypentanoic acid; N-Lactyl ethanolamine; |
| 443.1931          | 1.59   |            | 1-Hypoglycin A |
| 446.2394          | 7.21   |            | N-Acetylkadaverine; 1-3-Aminopropyl-4-aminobutanol |
| 448.1653          | 11.48  |            | 2-methyl-3,3-thiazolidine-2-carboxamide |
| 448.2181          | 7.47/7.10/7.92/9.43 |      | 2,6-diaminoethoxynic acid; L-Lysine; D-Lysine; |
| 451.1420          | 9.92   |            | 3,5-dihydroxy-3,4-dihydroxy-1,4-benzoathiazine |
| 451.1823          | 9.21/10.86/10.18 |      | 6-Methylenediamine; 1-Methylenediamine; 3-Methylenediamine; 7-Methylenediamine |
| 455.1773          | 8.94/6.60 |          | FAPy-adenine |
| 457.2821          | 6.34   |            | Propylhexedrine |
| 461.1794          | 11.16  |            | 1-Hexahydro-3-amino-1,2,4-oxadiazepine-3-carboxylic acid |
| 461.2858          | 8.26   |            | Aminopropylkadaverine; 4,4-Diaminodibutylamine |
| 462.1980          | 8.69   |            | D-Alanyl-D-alanine; Alanyl-Alanine; 4-Acetamidoo-2-aminobutanonic acid |
| 462.2334          | 5.23   |            | N(6)-Methyllysine; Isoputreanine |
| 464.1610          | 8.58   |            | 2-Indolecarboxylic acid; Indole-3-carboxylic acid; 3-Formyl-6-hydroxyindole |
| 464.2127          | 8.06   |            | 5-Hydroxylysine |
| 467.1591          | 8.37   |            | Methionine sulfoxide; Ethilin |
| 477.1373          | 5.74   |            | Guanidinosuccinic acid |
| 478.2072          | 5.07   |            | Serotonin |
| 479.1896          | 11.50  |            | O-Ureidohomoserine |
| 479.1920          | 11.84  |            | 5-Hydroxytryptophol; 2-Propanol 2-aminoazobenzene |
| 480.1644          | 8.12   |            | Melamine |
| 481.2080          | 11.97  |            | (R)-Salbutamol; alpha-Methylphenylalanine; N-methylphenylalanine; Salbutamol; |
| 482.1697          | 4.96   |            | Methionine sulfoximine |
| 482.2020          | 6.65   |            | 5-Hydroxykynurenamine; Tyrosinamide; L-2-Amino-3-(4-aminophenyl)propanoic acid |
| 483.1722          | 8.04   |            | 8-Hydroxy-7-methylxanthine |
| 487.1212          | 11.45  |            | Phosphoserine; DL-O-Phosphoserine |
| 493.1709          | 11.79  |            | 5-Hydroxyindolactic acid |
| 501.1728          | 51.68  |            | L-Epinephrine; L-Dopamine |
| 502.1647          | 10.22  |            | Spermine dialdehyde |
| 503.1482          | 4.92   |            | Thiabendazol |
| 507.1505          | 8.82   |            | 4,6-Dihydroxy-2-quinolinecarboxylic acid |
| 513.2364          | 11.23  |            | Methoxamine; Orceinanaline; Isoproterenol |

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Figure S4: The second 55 of the total 165 metabolites detected after analysis of fecal samples using the amine-specific chemical probe. Red indicates no detection; Green denotes detected metabolites, which are annotated on the basis of m/z value as an output from the XCMS analysis.

| Conjugates [M+H]^+ | RT/min | 6M 18M 21F | Annotation |
|---------------------|--------|------------|------------|
| 515.2499            | 11.24  |            | 2-nonenoylglycine |
| 516.1543            | 11.79  |            | Sulfacetamide |
| 517.1675            | 9.00/9.149/5.28/5.81/8.82 | Glycerylphosphorylthanolamine; sa-glycerol-3-Phosphoethanolamine |
| 518.1704            | 8.82/8.50/9.01 | 6-Aminopenicillinic acid |
| 520.1863            | 9.07/9.94 | Cysteinyl-Proline; Prolyl-Cysteine |
| 520.2027            | 10.18  |            | Glutamylalanine; gamma-Glutamylalanine; Hydroxyproplyl-Serine; |
| 520.2402            | 11.72  |            | Isoleucyl-Serine; Leucyl-Serine; Serylisoleucine; Serylleucine; |
| 521.1627            | 9.53   |            | L-Oxalylalbizziine |
| 523.2663            | 5.12   |            | Procarbazine |
| 523.9941            | 16.86  |            | Perchlorate |
| 536.1195            | 10.18/10.31 | Rihazole |
| 536.1806            | 8.47   |            | Cysteinyl-Hydroxypyroline; Hydroxyproplyl-Cysteine |
| 538.1158            | 9.93   |            | Brassinin |
| 545.1980            | 9.35/10.93/9.35 | Cytidine; Cytarabine; gamma-Glutamyl-beta-cyanoalanine |
| 545.2354            | 9.81   |            | Glutamylalanine; Prolyl-Glutamine; Prolyl-Gamma-glutamate |
| 547.1583            | 11.80  |            | Lamivudine sulfoxide |
| 547.2144            | 10.76/11.64/10.44 | Asparaginyl-Hydroxyproline; Hydroxyproplyl-Asparagine |
| 548.1729            | 10.45/10.46 | Methionyl-Proline; Prolyl-Methionine |
| 549.1575            | 10.64  |            | Emtricitabine |
| 551.1719            | 6.68/9.15/8.22 | Sulfapyridine |
| 552.1742            | 6.68/9.17 | gamma-Glutamylkysteine; Ghtamykysteine |
| 556.1838            | 9.86   |            | Acetaminophen cystein |
| 557.1289            | 10.91  |            | Sulfathiazole |
| 561.1922            | 6.67/9.68 | MinorBine |
| 561.2261            | 11.09  |            | Clobenorrex |
| 561.2291            | 11.26  |            | Glutamylhydroxyproline; Hydroxyproplyl-Glutamine; Hydroxyproplyl-Gamma-glutamate |
| 562.2495            | 6.51   |            | Gamma-Glutamylkystein; Glutamylkystein; Glutamylkystein; |
| 562.2784            | 9.03/8.93/9.39 | Glutaminylaspartic acid; Gltamylaspartic acid; gamma-Glutamylaspartic acid |
| 564.0426            | 5.26   |            | 3-phosphonotri-glyceroly phosphate; 3-phosphonotri-glyceroly phosphate; |
| 564.1929            | 12.08  |            | Glutamylaspartic acid; L-beta-asparyl-L-glutamic acid; gamma-Glutamylaspartic acid |
| 564.2127            | 9.05/9.69/9.38 | Hydroxyproplyl-Methionine; Methionyl-Hydroxyproline |
| 565.2809            | 8.36   |            | Protriptyline; Nortriptyline; demethylmaprotiline |
| 566.1918            | 7.99   |            | Asparyl-Methionine; Methionyl-Asparate; gamma-Glutamyl-S-methylkysteine |
| 570.1991            | 10.43  |            | Cysteinyl-Phenylalanine; Phenylalanyl-Cysteen |
| 576.2520            | 12.23  |            | Lysyl-Glutamate |
| 580.2061            | 6.67/6.40 | Glutamylmethionine; gamma-Glutamylmethionine |
| 581.2336            | 8.56   |            | Asparaginyl-phenylalanine; Phenylalanyl-Asparagine |
| 583.1356            | 12.84  |            | Ajosycysteine |
| 586.1946            | 9.38   |            | Cysteinyl-Tyroline; Tyrosyl-Cysteine |
| 587.1737            | 10.41  |            | Cladréine |
| 589.2208            | 10.92  |            | Rutacearpine |
| 589.2232            | 10.79/10.40/10.67 | N-Ribosylhistidine |
| 590.2415            | 13.20  |            | Imazamethabenz-oxymethyl |
| 591.2441            | 10.73/9.58 | Opithalamic acid; |
| 592.1657            | 9.38   |            | [1-(4,9-Dihydro-2-(methylthio)-1,3-thiazino[6,5-b]indol-4-yl)-2-propanone |
| 596.2171            | 10.90  |            | Dachtionic acid A; Dachtionic acid B |
| 601.1849            | 6.65   |            | Fenbendazol |
| 601.2470            | 5.97   |            | Sandoxin |
| 605.2370            | 10.86  |            | Indoleacetyl glutamine |
| 605.2593            | 12.20  |            | N5-Acetyl-N2-gamma-L-glutamyl-L-ornithine; Fenoterol; 6-Hydroxyetodolac; |
| 605.2599            | 12.35  |            | Fenoterol; 6-Hydroxyetodolac; 7-Hydroxyetodolac; alpha-noroxycodol; beta-noroxycodol |
| 610.1528            | 11.79  |            | Cytidine 2',3'-cyclic phosphate |
| 611.1241            | 11.79  |            | Lamivudine-monophosphate; 3-oxosaminolide |
| 613.1263            | 11.95  |            | 4'-Hydroxydiclofenac; 3'-Hydroxydiclofenac; 5'-Hydroxydiclofenac |
| 617.2589            | 12.24  |            | 2,5-dihydroxy-5-(methylamino)-3,4-diphenyketanoic acid |
Conjugates [M+H]+

| Conjugates | RT/min | 6M 18M21F | Annotation |
|------------|--------|-----------|-------------|
| 619.2333   | 10.15  | Red       | N-(1-Deoxy-1-fructosyl)histidine |
| 620.2721   | 13.62  | Red       | Fluvoxamine |
| 624.2492   | 12.62  | Red       | Avenic acid A; N2-Galacturonyl-L-lysine; N6-Galacturonyl-L-lysine |
| 626.1385   | 11.88  | Red       | Dorosolamide |
| 631.1635   | 10.45  | Red       | Cyclic AMP; Adenosine 2',3'-cyclic phosphate |
| 631.2735   | 13.23  | Red       | x-Anomuricine |
| 633.2501   | 9.16   | Red       | Ciprofloxacin |
| 644.2350   | 11.91  | Red       | Clozapine N-oxide |
| 650.2836   | 3.05   | Green     | Enalaprilat |
| 651.2245   | 8.62   | Green     | Ampicillin; Cefradine |
| 653.2501   | 13.27/13.22/12.59 | Red | Lomefloxacin |
| 653.3329   | 8.82   | Red       | Sphingosine 1-phosphate (d16:1-P) |
| 656.2338   | 11.06/9.32/9.17 | Red | Albutilamine |
| 665.1999   | 5.99   | Red       | Cefadroxil |
| 661.1700   | 9.17   | Red       | Fluorarabine; Metoxazone; Indapamide |
| 667.1715   | 9.17   | Red       | Metoxazone |
| 669.2656   | 7.97   | Yellow    | Tryptophyl-Tyrosine; Tyrosyl-Tryptophan |
| 676.2266   | 9.14   | Yellow    | Portulacoxanthin H |
| 677.2745   | 8.18/0.74 | Green | Guadroxacin |
| 681.2700   | 7.61/8.05 | Red | 2-(Arabinosylaminolino)-3-(glucosylaminolino)propanenitrile |
| 682.1223   | 5.76   | Yellow    | 4,11-Dichloro-5,12-dihydroquinolino[2,3-b]acridine-7,14-dione |
| 683.1920   | 11.78  | Yellow    | Cefadroxil |
| 684.1215   | 7.86   | Red       | Olomacine-O-sulfate |
| 685.2208   | 10.12/9.79 | Red | Succinyladenosine |
| 700.2545   | 8.10   | Red       | S-adenosyl-L-methioninate |
| 707.2162   | 10.24  | Red       | Cefalogycin |
| 710.2512   | 7.75   | Yellow    | Wybutoxine |
| 715.1277   | 11.77  | Yellow    | Halofuginone |
| 721.1469   | 5.76   | Red       | S-2,2-Dichloro-1-hydroxyethyl glutathione |
| 725.2273   | 12.05  | Yellow    | Gemcitabine diphosphate |
| 725.1331   | 8.69   | Yellow    | 5-Amino-4-hydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid |
| 726.1496   | 5.74   | Red       | Thiamine(1+); Diphostate(1-) |
| 729.1330   | 7.36/0.78 | Red | Adenosine phosphonurate |
| 730.1244   | 7.78   | Yellow    | IDP |
| 741.2527   | 4.73   | Red       | C26H31N3O9S |
| 741.3840   | 9.16   | Yellow    | LysyPE(0/0/15/0); LysyPE(15/0/0/0) |
| 750.2259   | 8.85   | Yellow    | Temocaprilat |
| 773.2654   | 11.51  | Red       | 10-Formyltetrahydrofolic acid; (6S)-5-formyltetrahydrofolic acid |
| 775.2574   | 12.05  | Red       | C26H31N3O9S |
| 777.1186   | 13.68  | Yellow    | Gemcitabine diphosphate |
| 781.3805   | 8.15   | Red       | PC-MS' |
| 782.2377   | 7.84   | Yellow    | Cefepime |
| 798.2439   | 6.77   | Red       | 1-Nitro-5-glutathionyl-6-hydroxy-5,6-dihydronaphthalene; |
| 805.4193   | 7.96   | Red       | LysyPE(0/0/15/14Z,17Z); LysyPE(0/0/20/3/5Z,8Z,11Z); |
| 825.1420   | 14.75/15.5/10.61 | Red | Cefadroxil |
| 833.2655   | 11.47  | Yellow    | Neoaeromicarine F; Neoaeromicarne I |
| 861.1871   | 10.47  | Yellow    | Adenosine diphosphate ribose |
| 863.2811   | 11.46  | Yellow    | C26H31N3O9S |
| 867.3680   | 7.27   | Yellow    | Hemorphin-4 |
| 889.1802   | 11.58  | Yellow    | GDP-4-Dehydro-6-deoxy-D-mannose; GDP-4-Dehydro-6-L-deoxygalactose |
| 895.2446   | 7.04   | Red       | C26H31N3O9S |
| 911.2816   | 11.54  | Red       | C26H31N3O9S |
| 914.2711   | 9.41   | Red       | Oxidized glutathione |
| 936.4078   | 7.26   | Yellow    | Recsinamine |
| 953.3232   | 11.72  | Yellow    | C26H31N3O9S |

**Figure S5:** The third 55 of the total 165 metabolites detected after analysis of fecal samples using the amine-specific chemical probe. Red indicates no detection; Green denotes detected metabolites, which are annotated on the basis of m/z value as an output from the XCMS analysis.
Figure S6: Representative EIC and mass spectrum for the conjugate of 1,5-diaminopentane in standard MS\textsuperscript{E} mode analysis. With a filter of m/z = 319 during our data analysis, we can exclude doubly annotated metabolites for charge states higher than 1.

3. Supporting tables

Table S1: LOD experiment in standard amines.

| Concentration | 1-Aminopropane | Piperidine | Aniline | L-Serine | 2-Amino-1-propanol |
|---------------|----------------|------------|---------|----------|--------------------|
| 100 µM        | 16.73          | 9.75       | 6.41    | ND       | 3.55               |
| 10 µM         | 0.78           | 1.41       | 3.14    | ND       | 1.04               |
| 1.0 µM        | ND             | ND         | ND      | ND       | ND                 |
| 100 nM        | ND             | ND         | ND      | ND       | ND                 |

Table S2: A list of the standard library synthesized from commercial amines in this study.

| Amine                     | Monoisotopic mass | Conjugates [M+H]\textsuperscript{+} | RT (min) |
|---------------------------|-------------------|--------------------------------------|----------|
| Ammonia                   | 17.0265           | 319.1401                             | 5.72     |
| Methylamine               | 31.0422           | 333.1558                             | 6.15     |
| Dimethylamine             | 45.0578           | 347.1714                             | 6.22     |
| Ethanolamine              | 61.0527           | 363.1663                             | 6.76     |
| 3-Aminopropanonitrile     | 70.0531           | 372.1667                             | 5.21     |
| 1-Amino-propan-2-ol       | 75.0684           | 377.1820                             | 6.40     |
| 2-(Methylamino) ethan-1-ol| 75.0684           | 377.1820                             | 6.00     |
| 3-Aminopropan-1-ol        | 75.0684           | 377.1820                             | 6.34     |
| Piperidine                | 85.0891           | 387.2027                             | 7.73     |
| 1,4 Diaminobutane         | 88.1000           | 390.2136                             | 5.22     |
| Alanine                   | 89.0476           | 391.1612                             | 6.80     |
| Aniline                   | 93.0578           | 395.1714                             | 9.84     |
| 1,5-Diaminopentane        | 102.1156          | 404.2292                             | 5.58     |
| 2-Aminoisobutyric acid    | 103.0633          | 405.1769                             | 5.09     |
| L-\(\alpha\)-aminobutyric acid | 103.0633    | 405.1769                             | 7.43     |
| y-Aminobutyric acid       | 103.0633          | 405.1769                             | 6.61     |
| Compound                        | Mass       | Retention Time | Purity     |
|--------------------------------|------------|---------------|------------|
| Diethanolamine                 | 242.0847   | 0.09          | 98.0       |
| Hypotaurine                    | 222.0034   | 0.09          | 98.0       |
| Histamine dihydrochloride      | 222.0034   | 0.09          | 98.0       |
| L-Valine                       | 117.0789   | 0.09          | 98.0       |
| 5-Aminovaleric acid            | 117.0789   | 0.09          | 98.0       |
| L-Ornithine                    | 117.0789   | 0.09          | 98.0       |
| L-Homoserine                   | 119.0582   | 0.09          | 98.0       |
| L-Threonine                    | 119.0600   | 0.09          | 98.0       |
| α-Methylbenzylamine            | 121.0891   | 0.09          | 98.0       |
| 2,6-Dimethoxyline              | 121.0891   | 0.09          | 98.0       |
| Phenethylamine                 | 121.0891   | 0.09          | 98.0       |
| 5-Methylcytosine               | 125.0589   | 0.09          | 98.0       |
| L-(-)-Pipelicolic acid         | 129.0789   | 0.09          | 98.0       |
| L-Hydroxyproline               | 131.0582   | 0.09          | 98.0       |
| L-Isoleucine                   | 131.0946   | 0.09          | 98.0       |
| L-Norleucine                   | 131.0946   | 0.09          | 98.0       |
| 6-Aminocaproic acid            | 131.0946   | 0.09          | 98.0       |
| Glycol-glycine                 | 132.0534   | 0.09          | 98.0       |
| D-Aspartic acid                | 133.0375   | 0.09          | 98.0       |
| DL-Homocysteine                | 135.0354   | 0.09          | 98.0       |
| Adenine                        | 135.0544   | 0.09          | 98.0       |
| 4-Aminobenzoic acid            | 137.0476   | 0.09          | 98.0       |
| O-Phosphoryl-ethanolamine      | 141.0190   | 0.09          | 98.0       |
| L-Histidinol                   | 141.0902   | 0.09          | 98.0       |
| N-(S-Aminopentyl) acetamide    | 144.1262   | 0.09          | 98.0       |
| Spermidine                     | 145.1578   | 0.09          | 98.0       |
| L-Lysine                       | 146.1055   | 0.09          | 98.0       |
| L-Glutamine                    | 146.1400   | 0.09          | 98.0       |
| L-Glutamic acid                | 147.0531   | 0.09          | 98.0       |
| Phenylglycine                  | 151.0633   | 0.09          | 98.0       |
| N,N-Dimethylamine HCl          | 151.0997   | 0.09          | 98.0       |
| 5-Aminosalicylic acid          | 153.0425   | 0.09          | 98.0       |
| DL-2-Aminocaproic acid         | 159.1259   | 0.09          | 98.0       |
| Tryptamine                     | 160.1000   | 0.09          | 98.0       |
| S-Allyl-L-cysteine             | 161.0511   | 0.09          | 98.0       |
| N,N-Dimethyl-L-glutamic acid   | 161.0688   | 0.09          | 98.0       |
| Aminoadipic acid               | 161.0700   | 0.09          | 98.0       |
| DL-5-Hydroxylsine              | 162.1004   | 0.09          | 98.0       |
| 1-Methylguanidine              | 165.0650   | 0.09          | 98.0       |
| Pyridoxamine                   | 168.0898   | 0.09          | 98.0       |
| L-Cysteic acid                 | 169.0044   | 0.09          | 98.0       |
| 3-Methyl-L-histidine           | 169.0851   | 0.09          | 98.0       |
| Glycolvaline                   | 174.1004   | 0.09          | 98.0       |
| Glucosamine hydrochloride      | 180.0872   | 0.09          | 98.0       |
| N-acetyl-N-L-lysine            | 188.1160   | 0.09          | 98.0       |
| Glycol-L-leucine               | 188.1160   | 0.09          | 98.0       |
| N-acetyl-L-lysine              | 188.1161   | 0.09          | 98.0       |
| L-Homoarginine                 | 188.1273   | 0.09          | 98.0       |
| L-homocitrulline               | 189.1113   | 0.09          | 98.0       |
| 2,6-Diaminopimelic Acid        | 190.0953   | 0.09          | 98.0       |
| Leucyl-Alanine                 | 202.1317   | 0.09          | 98.0       |
| L-Tryptophan                   | 204.0898   | 0.09          | 98.0       |
| D-(-)-tryptophan               | 204.0898   | 0.09          | 98.0       |
| L-Kynurenine                   | 208.0847   | 0.09          | 98.0       |
| L-Alanyl-L-glutamine           | 217.1063   | 0.09          | 98.0       |
| L-5-Hydroxytryptophan          | 220.0847   | 0.09          | 98.0       |
| L-Carnosine                    | 226.1066   | 0.09          | 98.0       |
| L-Alanyl-L-phenylalanine       | 236.1161   | 0.09          | 98.0       |
| Glycol-L-tyrosine              | 238.0953   | 0.09          | 98.0       |
| L-Cystine                      | 240.0238   | 0.09          | 98.0       |
| Triamterene                    | 253.1075   | 0.09          | 98.0       |
| 5-Methylcytidine               | 257.1012   | 0.09          | 98.0       |
| Thiamine                      | 265.1123   | 0.09          | 98.0       |
| Atenolol                       | 266.1630   | 0.09          | 98.0       |
| 2'-Deoxyguanosine              | 267.1000   | 0.09          | 98.0       |
| Guanosine                      | 283.0916   | 0.09          | 98.0       |
| Hydrochlorothiazide            | 296.8644   | 0.09          | 98.0       |
| Furosemide                     | 330.0077   | 0.09          | 98.0       |
| Adenosine-5'-monophosphate     | 347.0630   | 0.09          | 98.0       |
| Folinic acid calcium salt hydrat | 471.1503   | 0.09          | 98.0       |
| Cytidine 5'-triphosphate       | 482.9845   | 0.09          | 98.0       |
| Adenosine 5'-triphosphate      | 506.9957   | 0.09          | 98.0       |
| Guanosine 5'-triphosphate      | 522.9907   | 0.09          | 98.0       |
| NAD                            | 664.1169   | 0.09          | 98.0       |
| S-(5'-Adenosyl)-L-methionine   | 399.1450   | 0.09          | 98.0       |
Table S3: A list of validated metabolites and their associated diseases.

| HMDB ID     | Metabolites                | Disease                    |
|-------------|----------------------------|----------------------------|
| HMDB0000051 | Ammonia                    | Short bowel syndrome       |
| HMDB000164  | Methylamine                | Crohn's disease            |
| HMDB000087  | Dimethylamine              | Pancreatic cancer          |
| HMDB003401  | Piperidine                 | Colorectal cancer          |
| HMDB001414  | 1,4-Diaminobutane          | Alzheimer's disease        |
| HMDB000161  | L-Alanine                  | Colorectal cancer          |
| HMDB001310  | D-Alanine                  | Early preeclampsia         |
| HMDB002322  | 1,5-Diaminopentane         | Colorectal cancer          |
| HMDB000452  | L-α-aminobutyric acid      | Alzheimer's disease        |
| HMDB000112  | γ-Aminobutyric acid        | Alzheimer's disease        |
| HMDB000883  | L-Valine                   | Schizophrenia              |
| HMDB003355  | 5-Aminovaleric acid        | Irritable bowel syndrome   |
| HMDB000167  | L-Threonine                | Heart failure              |
| HMDB001275  | 2-Phenethylamine           | Crohn's disease            |
| HMDB000070  | Pipecolic acid             | Colorectal cancer          |
| HMDB000725  | L-Hydroxyproline           | Alzheimer's disease        |
| HMDB000172  | L-Isoleucine               | Heart failure              |
| HMDB000742  | DL-Homocysteine            | Stroke                     |
| HMDB001392  | 4-Aminobenzoic acid        | Colorectal cancer          |
| HMDB000224  | O-Phosphoryl-ethanolamine  | Crohn's disease            |
| HMDB002284  | N-(5-Aminopentyl) acetamide| Colorectal cancer          |
| HMDB000182  | L-Lysine                   | Schizophrenia              |
| HMDB000641  | L-Glutamine                | Colorectal cancer          |
| HMDB003423  | S-Ally-L-cysteine          | Alzheimer's disease        |
| HMDB000450  | DL-5-Hydroxylysine         | Colorectal cancer          |
| HMDB0028854 | Glycylvaline               | Colorectal cancer          |
| HMDB000446  | N-α-Acetyl-L-lysine        | Colorectal cancer          |
| HMDB000472  | L-5-Hydroxytryptophan      | Ulcerative colitis         |
| HMDB000033  | L-Carnosine                | Alzheimer's disease        |
| HMDB000235  | Thiamine                   | Hemodialysis               |
| HMDB000133  | Guanosine                  | Colorectal cancer          |
4. General

All non-aqueous reactions were performed using flame- or oven dried glassware under an atmosphere of dry nitrogen. All reagents and solvents were purchased from Sigma-Aldrich or Fischer Scientific and were used without further purification. The in-house built metabolite library was obtained from MetaSci. Mass spectrometry grade solvents were used for UHPLC-ESI-MS analysis. Solutions were concentrated in vacuo on a Heidolph or a IKA rotary evaporator. Thin Layer Chromatography (TLC) was performed on silica gel 60 F-254 plates. Visualization of the developed chromatogram was performed using fluorescence quenching. Chromatographic purification of products was accomplished using flash column chromatography on Merck silica gel 60 (40–63 μm). All synthesized compounds were ≥95% pure as determined by NMR. NMR spectra were recorded on Agilent 400 MHz spectrometer (1H NMR: 399.97 MHz, 13C NMR: 100.58 MHz). Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Glass vials used for handling magnetic beads were microwave vials from Biotage (0.2–0.5 mL or 0.5–2.0 mL). High-resolution mass spectra were acquired on a SYNAPT G2-S High-Definition Mass Spectrometer (HDMS) using an electrospray ionization (ESI) source with an ACQUITY UPLC I-class system and equipped with a Waters ACQUITY UPLC BEH C18 column (2.1 × 75 mm, 1.7 μm particle size) for chemoselective-probe based metabolomics analysis or Waters ACQUITY UPLC HSS T3 column (1.8 × 100 mm, 2.1 μm particle size) for compound characterization of compound 6. The mobile phase consisted of a combination of 0.1% formic acid in MilliQ water (A) and 0.1% formic acid in LC-MS grade methanol (B). The column temperature was 40 °C and the mobile phase gradient applied was as follows: 0-2 min, 0% B; 2-15 min, 0-100% B; 15-18 min, 100% B; 18-20 min, 100-0% B; 20-25 min, 0% B, with a flow rate of 0.3 mL/min.

The samples were introduced into the q-TOF using positive electrospray ionization. The capillary voltage was set to -2.50 kV and the cone voltage was 40 V. The source temperature was 100 °C, the cone gas flow 50 L/min and the desolvation gas flow 600 L/h. The instrument was operated in MSE mode, the scan range was m/z = 50-1200, and the scan time was 0.3 s. A solution of sodium formate (0.5 mM in 2-propanol: water, 90:10, v/v) was used to calibrate the instrument and a solution of leucine-encephalin (2 ng/µl in acetonitrile: 0.1% formic acid in water, 50:50, v/v) was used for the lock mass correction at an injection rate of 30 s.

All chemical synthesis protocols and characterization data are available in the Supplementary Information.
5. Description of procedures

5.1 Preparation of bead-bound, unactivated probe 1

MagnaBind Amine Derivatized Beads slurry (50 µL, 320 nmol, Thermo ScientificTM) was transferred into a 1.5 mL Eppendorf tube. Original solution from supplier was taken out by magnetic separation. The beads were washed with THF (2×150 µL) followed by phosphate buffer (2×150 µL, 25 nM, pH 7.5). DMF (150 µL) was added to the Eppendorf followed by 5 µL DIPEA and then vortexed for at least 30 s to yield the unprotonated amine. The beads were washed with DMF (150 µL) followed by DCM (150 µL). An amide coupling solution (4.5 mM PyBop, 3.3 mM HOBT, 1% DIPEA v/v in DCM) and probe solution (3 mM probe in DMF) were freshly prepared as reported previously in separate1. The probe solution (100 µL) and amide coupling solution (100 µL) were combined into the Eppendorf tube containing magnetic beads. The mixture was shaken and incubated using a Thermomixer (1,600 rpm, 25 °C, overnight.). The solution was removed and the beads consecutively washed with 2×150 µL THF and 2×150 µL DCM. After removal of all the solution, DCM (190 µL) and TFA (10 µL) were added in sequence to the Eppendorf for Boc deprotection. The mixture was shaken and incubated with a Thermomixer (1,500 rpm, 25 °C, 5 h). The reaction mixture was removed and followed by washing with THF (2×150 µL). DCM (150 µL) and DIPEA (10 µL) were added in sequence to the Eppendorf for amine deprotonation and TFA neutralization. The beads were washed with DMF (2×150 µL) and EtOH (2×150 µL). The beads were suspended in the EtOH (300 µL), ready to be used for chemoselective probe activation.

5.2 Activation of amine-specific chemoselective probe 2

The bead-bound, unactivated probe 1 (in 300 µL EtOH) were added with 3,4-Diethoxy-3-cyclobutene-1,2-dione (5 µL) and trimethylamine (3 µL). The suspension was agitated at 25 °C in a ThermoMixer (1,600 rpm) for 16 h. After the reaction was complete, the supernatant was removed and the beads were washed with THF (3×200 µL) followed by EtOH (2×200 µL). The beads were suspended in the EtOH (300 µL), ready to be used for sample treatment.

5.3 Preparation of fecal metabolite extracts

A scalpel was used to collect approximately 30 mg of the frozen fecal sample from ten different patients (stored at 80 °C) in specialized tube D (MP Biomedicals). Ultrapure water (50 µL) and LCMS grade methanol (200 µL) were added into each tube. The mixture was vortexed and subsequently homogenized by a FastPrep 24 homogenizer (3 cycles, 6 m/s, 40 s, MP Biomedicals). The mixture was taken out from tube D into Eppendorf tubes and stored at -20 °C for at least 1 h for protein precipitation. The supernatant was collected after centrifugation (18,620 g, 5 min, 4 °C). The extracts were direct used in the bead treatment.

5.4 Treatment of fecal metabolite extracts

The activated beads 2 were used to treat the fecal extract in a solution of 1% v/v trimethylamine in ethanol. The mixture was shaken for 16 h at 1500 rpm and 55 °C. The fecal extract solution was removed from the beads and the beads were washed with THF (2×200 µL) before being resuspended in THF (300 µL).
5.5 Cleavage of the bead-bound chemical probe 3

The suspension of beads was transferred to a glass vial. Triphenylphosphine (97.0 µL, 12.9 mM in THF, 1.25 µmol) and dimethylbarbituric acid (90.0 µL, 30.7 mM in THF, 2.76 µmol) solutions were added to the vial, followed by palladium (II) acetate solution (84.0 µL, 6.53 mM in THF, 549 nmol). The vial was quickly sealed and a stream of nitrogen was passed through until approximately half the volume of the suspension remained. The vial was agitated at intervals on a vortexer and the reaction was allowed to continue 5 h. In parallel, a sample of unmodified beads was treated with the same cleavage conditions as the activated beads treated with fecal extract and used as control sample. The supernatant was removed from the beads using magnetic separation and the solvent removed using a vacuum centrifuge. The residues were redisolved in MeOH (30 µL each) and triphenylphosphine and triphenylphosphate oxide were precipitated through the addition of water (120 µL each). The suspension was centrifuged (benchtop centrifuge, 13,400 rpm, 5 min), the supernatant removed, and the solvent was again removed with the vacuum centrifuge. The residues were redisolved in water/acetonitrile solution (95:5 v/v) and submitted for LC-MS analysis.

5.6 Synthesis of Fmoc-protected simplified probe 6

![Diagram of compounds 5 and 6]

Compound 5 (4.9 mg, 11.0 nmol), 3,4-diethoxy-3-cyclobutene-1,2-dione (squaric acid diethyl ester, 3.7 mg, 22.0 nmol) and triethylamine (3.3 mg, 33.0 nmol) were combined with ethanol (2.0 ml) in a microwave tube. The tube was sealed and stirred at 45 °C for 5 hours. After the complete consumption of the compound 5 by monitoring TLC, the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel using a gradient of 1-5% MeOH in DCM to yield compound 6 as white solid (5.2 mg, 83.0%).

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1H NMR (400 MHz, CDCl₃) δ 7.76 (dd, J = 21.5, 7.9 Hz, 4H, Fmoc Ar), 7.51 (d, J = 7.5 Hz, 2H, Ph), 7.38 (tt, J = 7.5, 0.9 Hz, 2H, Ph), 7.31 – 7.09 (m, 4H, Fmoc Ar), 6.74 (s, 1H, amide), 5.21 (s, 1H, amide), 4.78 (q, J = 7.1 Hz, 2H, OCH₂CH₃), 4.28 (d, J = 7.2 Hz, 2H, FmocCH₂), 4.13 (s, 1H, FmocCH₂), 3.70 – 3.62 (m, 4H, ArCONHCH₂CH₂FmocNHCH₂CH₂), 3.61 – 3.55 (m, 2H, ArCONHC₂H₂), 3.44 – 3.40 (m, 2H, FmocNHCH₂), 2.80 (s, 1H, aniline), 1.44 (t, J = 7.1 Hz, 3H, OCH₂CH₃). 13C NMR (101 MHz, CDCl₃) δ 184.4, 166.9, 156.9, 144.0, 141.4, 139.8, 130.5, 128.8, 127.9, 127.2, 125.2, 120.2, 118.6, 114.3, 70.7, 70.4, 69.5, 67.0, 47.3, 40.8, 40.1, 16.0. HRMS (ESI+) m/z [M+H]+ calcd. for C₃₂H₃₂O₇N₅+: 570.2235; found 570.2246.

5.7 Preparation of probe-conjugated standards for LOD measurement

A solution of Fmoc-protected probe 6 (10 µL, 10.0 mM in EtOH, 100 nmol, Scheme S2) was evaporated under reduced pressure. The residue was then combined with a solution of single amine standard (40 µL, 2 equiv., 5.0 mM in EtOH), 3 µL trimethylamine and 147 µL ethanol. The resulting solution was then shaken at 1600 rpm for 16 h at 55 °C. The solvents were then removed under reduced pressure, and the residues were treated with piperidine (80 µL) and shaken at 1600 rpm for 5 h at 25 °C. The piperidine was then removed under reduced pressure, and the residue was redissolved in EtOH (200 µL). The solution was diluted in a series of
concentration (100 µL, 10 µL, 1 µL, 100nM, 10 nM, 1 nM, 0.1 nM, 0.08 nM, 0.05nM and 0.01 nM) in a solution of water and acetonitrile (95:5 v/v) before being submitted for UPLC-MS analysis. In parallel, the amine standards have also been prepared in the concentration mentioned above for investigation of the improvement of the sensitivity.

5.8 Reactivity test experiment

For testing the reactivity of Fmoc-protected probe 6 for different amine metabolite classes. Herein, 1L of 4-methylmorpholine/acetic acid buffer solution was prepared in different pH (7.5, 8.5, and 9.5).

The Fmoc-protected probe 6 and amines conjugation was performed according to section 6.7 with minor modification. The probe 6 (50 µL, 10.0 mM in EtOH) was mixed with 1-aminopropane (primary amine, 8.33 µL, 10.0 mM in EtOH), piperidine (secondary amine, 8.33 µL, 10.0 mM in EtOH), aniline (aromatic amine, 8.33 µL, 10.0 mM in EtOH), L-serine (amino acid, 8.33 µL, 10.0 mM in EtOH), and N-acetyl-L-cysteine (thiol, 8.33 µL, 10.0 mM in EtOH) in 200 µL different buffer solution including pH 7.5, 8.5, 9.5 and ethanol with 1% trimethylamine for 16 hours at 55 °C. The solvents were then removed under reduced pressure, and the residues were treated with piperidine (80 µL) and shaken at 1600 rpm for 5 h at 25 °C. The piperidine was then removed under reduced pressure, and the residue was redissolved in MeOH (100 µL) followed by water (400 µL). The solution was diluted as necessary in a solution of water and acetonitrile (95:5 v/v) before being submitted for UPLC-MS analysis.

5.9 Stability test experiment.

The synthetic conjugated metabolites in section 6.8 were treated in the same condition as biorthogonal cleavage in section 6.5 for 16 hours.

5.10 Construction of amine-containing metabolite library

The library construction was performed by following the same procedure (section 6.4; 6.5) as human sample treatment replacing the human metabolite extracts into amine metabolite standards. Single injection was submitted to UPLC-MS.

5.11 LC-MS analysis

Four injections were performed for fecal extract-treated bead cleavage product and six injections for the control sample. For the first 90 s of the analysis, the output of the UHPLC system was diverted to waste and did not enter the mass spectrometer.

5.12 Data analysis

Data files from the LC-MS analysis were converted into the NetCDF file format using MassLynx 4.1 (Waters). The XCMS library was used to perform peak detection and align the chromatograms. The feature list was reduced by eliminating those features with an m/z value less than 319.1401 (the m/z value corresponding to the monoprotonated probe with ammonium conjugate). More abundant features in the control sample and less than five-fold higher abundance in the feces sample set were eliminated from the data analysis. The features that eluted earlier than 1.5 min were also removed. Mass values of each feature with 302.1136 Da subtracted (corresponding to the mass of the probe) were compared to the human metabolome
database in order to find plausible candidates for the parent metabolites. Commercial or synthetic standards (section 6.10) were then used to confirm the identity of the metabolites and identification of the correct regioisomers.

6. Reference

1. N. Garg, L. P. Conway, C. Ballet, M. S. P. Correia, F. K. S. Olsson, M. Vujasinovic, J. M. Lohr and D. Globisch, Angew. Chem., Int. Ed., 2018, 57, 13805-13809.

7. NMR Spectra