

# High-Resolution NMR Techniques in Organic Chemistry

---

Third Edition

**Timothy D.W. Claridge**  
Chemistry Research Laboratory  
Department of Chemistry  
University of Oxford  
Oxford, United Kingdom



AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS  
SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

# Contents

|   |    |   |     |
|---|----|---|-----|
| Preface   | ix | 2.6.2 Lineshape Analysis and Thermodynamic Parameters       | 53  |
|   |    | 2.6.3 Magnetisation Transfer under Slow-Exchange Conditions | 55  |
|   |    | References  | 58  |
| <b>1 Introduction</b>   |    | <b>3 Practical Aspects of High-Resolution NMR</b>           |     |
| 1.1 The Development of High-Resolution NMR                                | 1  | 3.1 An Overview of the NMR Spectrometer                     | 61  |
| 1.2 Modern High-Resolution NMR and This Book                              | 3  | 3.2 Data Acquisition and Processing                         | 64  |
| 1.2.1 What This Book Contains   | 4  | 3.2.1 Pulse Excitation                                      | 64  |
| 1.2.2 Pulse Sequence Nomenclature   | 5  | 3.2.2 Signal Detection                                      | 66  |
| 1.3 Applying Modern NMR Techniques  | 7  | 3.2.3 Sampling the FID                                      | 67  |
| References  | 10 | 3.2.4 Quadrature Detection                                  | 73  |
|   |    | 3.2.5 Phase Cycling   | 78  |
|   |    | 3.2.6 Dynamic Range and Signal Averaging                    | 80  |
| <b>2 Introducing High-Resolution NMR</b>                                  |    | 3.2.7 Window Functions                                      | 83  |
| 2.1 Nuclear Spin and Resonance  | 11 | 3.2.8 Phase Correction                                      | 88  |
| 2.2 The Vector Model of NMR   | 14 | 3.3 Preparing the Sample                                    | 89  |
| 2.2.1 The Rotating Frame of Reference                                     | 14 | 3.3.1 Selecting the Solvent                                 | 89  |
| 2.2.2 Pulses  | 15 | 3.3.2 Reference Compounds                                   | 91  |
| 2.2.3 Chemical Shifts and Couplings                                       | 17 | 3.3.3 Tubes and Sample Volumes                              | 92  |
| 2.2.4 Spin-Echoes   | 18 | 3.3.4 Filtering and Degassing                               | 94  |
| 2.3 Time and Frequency Domains  | 20 | 3.4 Preparing the Spectrometer                              | 95  |
| 2.4 Spin Relaxation   | 22 | 3.4.1 The Probe   | 95  |
| 2.4.1 Longitudinal Relaxation: Establishing Equilibrium                   | 22 | 3.4.2 Probe Design and Sensitivity                          | 97  |
| 2.4.2 Measuring $T_1$ with the Inversion Recovery Sequence                | 24 | 3.4.3 Tuning the Probe                                      | 103 |
| 2.4.3 Transverse Relaxation: Loss of Magnetisation in the $x$ - $y$ Plane | 26 | 3.4.4 The Field Frequency Lock                              | 105 |
| 2.4.4 Measuring $T_2$ with a Spin-Echo Sequence                           | 27 | 3.4.5 Optimising Field Homogeneity: Shimming                | 107 |
| 2.5 Mechanisms for Relaxation   | 31 | 3.4.6 Reference Deconvolution                               | 112 |
| 2.5.1 The Path to Relaxation  | 32 | 3.5 Spectrometer Calibrations                               | 113 |
| 2.5.2 Dipole-Dipole Relaxation  | 33 | 3.5.1 Radiofrequency Pulses                                 | 113 |
| 2.5.3 Chemical Shift Anisotropy Relaxation                                | 34 | 3.5.2 Pulsed Field Gradients                                | 122 |
| 2.5.4 Spin Rotation Relaxation  | 35 | 3.5.3 Sample Temperature                                    | 124 |
| 2.5.5 Quadrupolar Relaxation  | 35 | 3.6 Spectrometer Performance Tests                          | 126 |
| 2.6 Dynamic Effects in NMR  | 38 | 3.6.1 Lineshape and Resolution                              | 127 |
| 2.6.1 The Influence of Dynamic Exchange                                   | 39 | 3.6.2 Sensitivity   | 128 |
|   |    | 3.6.3 Solvent Presaturation                                 | 130 |
|   |    | References  | 130 |

|          |  |     |          |   |     |
|----------|--|-----|----------|---|-----|
| <b>4</b> | <b>One-Dimensional Techniques</b>                                |     | <b>6</b> | <b>Correlations Through the Chemical Bond I: Homonuclear Shift Correlation</b>    |     |
| 4.1      | Single-Pulse Experiment  | 133 | 6.1      | Correlation Spectroscopy: COSY  | 203 |
| 4.1.1    | Optimising Sensitivity   | 133 | 6.1.1    | Interpreting COSY   | 204 |
| 4.1.2    | Quantitative NMR Measurements and Integration                    | 137 | 6.1.2    | Peak Fine Structure   | 207 |
| 4.1.3    | Quantification with an Electronic Calibrant: ERETIC              | 140 | 6.1.3    | Which COSY Approach?  | 210 |
| 4.1.4    | Quantification with an External Calibrant: PULCON                | 142 | 6.1.4    | COSY- $\beta$   | 211 |
| 4.2      | Spin-Decoupling Methods  | 143 | 6.1.5    | Double-Quantum Filtered COSY (DQF-COSY)   | 212 |
| 4.2.1    | Basis of Spin Decoupling   | 143 | 6.1.6    | Long-Range COSY: Detecting Small Couplings  | 219 |
| 4.2.2    | Homonuclear Decoupling   | 143 | 6.1.7    | Relayed-COSY  | 220 |
| 4.2.3    | Heteronuclear Decoupling   | 145 | 6.2      | Total Correlation Spectroscopy: TOCSY   | 220 |
| 4.3      | Spectrum Editing with Spin-Echoes                                | 148 | 6.2.1    | The TOCSY Sequence  | 221 |
| 4.3.1    | J-Modulated Spin-Echo  | 149 | 6.2.2    | Applying TOCSY  | 223 |
| 4.3.2    | APT  | 152 | 6.2.3    | Implementing TOCSY  | 225 |
| 4.4      | Sensitivity Enhancement and Spectrum Editing                     | 153 | 6.2.4    | One-Dimensional TOCSY   | 227 |
| 4.4.1    | Polarisation Transfer  | 154 | 6.3      | Correlating Dilute Spins: INADEQUATE  | 230 |
| 4.4.2    | INEPT  | 156 | 6.3.1    | Two-Dimensional INADEQUATE  | 230 |
| 4.4.3    | DEPT   | 162 | 6.3.2    | One-Dimensional INADEQUATE  | 233 |
| 4.4.4    | DEPTQ  | 165 | 6.3.3    | Implementing INADEQUATE   | 233 |
| 4.5      | Observing Quadrupolar Nuclei                                     | 167 | 6.4      | Correlating Dilute Spins Via Protons: ADEQUATE                                    | 235 |
|          | References   | 168 | 6.4.1    | Two-Dimensional ADEQUATE  | 236 |
|          |  |     | 6.4.2    | Enhancements to ADEQUATE  | 237 |
|          |  |     |          | References  | 240 |
| <b>5</b> | <b>Introducing Two-Dimensional and Pulsed Field Gradient NMR</b> |     | <b>7</b> | <b>Correlations Through the Chemical Bond II: Heteronuclear Shift Correlation</b> |     |
| 5.1      | Two-Dimensional Experiments                                      | 172 | 7.1      | Introduction  | 243 |
| 5.1.1    | Generating the Second Dimension                                  | 172 | 7.2      | Sensitivity   | 244 |
| 5.1.2    | Correlating Coupled Spins  | 176 | 7.3      | Heteronuclear Single-Bond Correlations  | 246 |
| 5.2      | Practical Aspects of 2D NMR                                      | 177 | 7.3.1    | Heteronuclear Single-Quantum Correlation  | 246 |
| 5.2.1    | Two-Dimensional Lineshapes and Quadrature Detection              | 177 | 7.3.2    | Hybrid HSQC Experiments   | 253 |
| 5.2.2    | Axial Peaks  | 181 | 7.3.3    | Heteronuclear Multiple-Quantum Correlation  | 257 |
| 5.2.3    | Instrumental Artefacts   | 182 | 7.4      | Heteronuclear Multiple-Bond Correlations  | 261 |
| 5.2.4    | Two-Dimensional Data Acquisition                                 | 183 | 7.4.1    | HMBC Sequence   | 263 |
| 5.2.5    | Two-Dimensional Data Processing                                  | 186 | 7.4.2    | Applying HMBC   | 264 |
| 5.3      | Coherence and Coherence Transfer                                 | 188 | 7.4.3    | HMBC Extensions and Variants  | 266 |
| 5.3.1    | Coherence Transfer Pathways                                      | 190 | 7.4.4    | H2BC: Differentiating $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ HMBC Correlations   | 274 |
| 5.4      | Gradient-Selected Spectroscopy                                   | 191 | 7.4.5    | Measuring Long-Range $^nJ_{\text{XH}}$ Coupling Constants                         | 275 |
| 5.4.1    | Signal Selection with Pulsed Field Gradients                     | 192 | 7.4.6    | Long-Range HSQMBC: Interrogating Proton-Sparse Molecules                          | 281 |
| 5.4.2    | Phase-Sensitive Experiments: <i>Echo–Antiecho</i> Selection      | 195 | 7.5      | Heteronuclear X-Detected Correlations   | 282 |
| 5.4.3    | Pulsed Field Gradients in High-Resolution NMR                    | 196 | 7.5.1    | Single-Bond Heteronuclear Correlations  | 283 |
| 5.4.4    | Practical Implementation of Pulsed Field Gradients               | 198 | 7.5.2    | Multiple-Bond Correlations and Small Couplings                                    | 285 |
| 5.4.5    | Fast Data Acquisition: Single-Scan Two-Dimensional NMR           | 199 |          |   |     |
|          | References   | 201 |          |   |     |

|                |  |     |           |   |     |
|----------------|--|-----|-----------|---|-----|
| 7.6            | <b>Heteronuclear X–Y Correlations</b>  | 286 | 9.7       | <b>Measuring Rotating Frame<br/>NOEs: ROESY</b>             | 353 |
| 7.6.1          | Direct X–Y Correlations  | 286 | 9.7.1     | The 2D ROESY Sequence                                       | 353 |
| 7.6.2          | Indirect <sup>1</sup> H-Detected<br>X–Y Correlations                               | 288 | 9.7.2     | 1D ROESY Sequences  | 355 |
| 7.7            | <b>Parallel Acquisition NMR with Multiple<br/>Receivers</b>                        | 291 | 9.7.3     | Applications  | 356 |
|                | References   | 292 | 9.8       | <b>Measuring Steady-State NOEs: NOE<br/>Difference</b>      | 359 |
| <b>8</b>       | <b>Separating Shifts and Couplings:<br/>J-Resolved and Pure Shift Spectroscopy</b> |     | 9.8.1     | Optimising Difference Experiments                           | 361 |
| 8.1            | <b>Introduction</b>  | 295 | 9.9       | <b>Measuring Heteronuclear NOEs:<br/>HOESY</b>              | 363 |
| 8.2            | <b>Heteronuclear J-Resolved Spectroscopy</b>                                       | 295 | 9.9.1     | 2D Heteronuclear NOEs                                       | 364 |
| 8.2.1          | Measuring Long-Range<br>Proton–Carbon Coupling<br>Constants                        | 298 | 9.9.2     | 1D Heteronuclear Nuclear<br>Overhauser Effects              | 365 |
| 8.2.2          | Practical Considerations   | 300 | 9.9.3     | Applications  | 366 |
| 8.3            | <b>Homonuclear J-Resolved Spectroscopy</b>   | 301 | 9.10      | <b>Experimental Considerations<br/>for NOE Measurements</b> | 367 |
| 8.3.1          | Tilting, Projections<br>and Symmetrisation   | 302 | 9.11      | <b>Measuring Chemical Exchange: EXSY</b>                    | 368 |
| 8.3.2          | Applications   | 303 | 9.12      | <b>Residual Dipolar Couplings</b>                           | 371 |
| 8.4            | <b>'Indirect' Homonuclear J-Resolved<br/>Spectroscopy</b>                          | 304 | 9.12.1    | Measuring RDCs  | 372 |
| 8.5            | <b>Pure Shift Broadband-Decoupled<br/><sup>1</sup>H Spectroscopy</b>               | 306 | 9.12.2    | Applying RDCs   | 375 |
| 8.5.1          | The Basis of Pure Shift<br>Spectroscopy  | 307 |           | References  | 377 |
| 8.5.2          | Pseudo-2D Pure Shift   | 307 | <b>10</b> | <b>Diffusion NMR Spectroscopy</b>                           |     |
| 8.5.3          | Real-Time Pure Shift   | 309 | 10.1      | <b>Introduction</b>   | 381 |
| 8.5.4          | Pure Shift Refocussing Elements  | 309 | 10.1.1    | Diffusion Coefficients<br>and Molecular Size                | 382 |
|                | References   | 313 | 10.2      | <b>Measuring Self-Diffusion by NMR</b>                      | 382 |
| <b>9</b>       | <b>Correlations Through Space: The Nuclear<br/>Overhauser Effect</b>               |     | 10.2.1    | The Pulsed Field Gradient<br>Spin-Echo                      | 383 |
| 9.1            | <b>Introduction</b>  | 315 | 10.2.2    | The Pulsed Field Gradient<br>Stimulated-Echo                | 384 |
| <b>PART I</b>  | <b>THEORETICAL ASPECTS</b>   | 317 | 10.2.3    | Enhancements to the<br>Stimulated-Echo                      | 385 |
| 9.2            | <b>Definition of the NOE</b>   | 317 | 10.2.4    | Data Analysis: Regression Fitting                           | 388 |
| 9.3            | <b>Steady-State NOEs</b>   | 317 | 10.2.5    | Data Analysis: Pseudo-2D<br>Presentation                    | 389 |
| 9.3.1          | NOEs in a Two-Spin System  | 317 | 10.3      | <b>Practical Aspects of Diffusion NMR<br/>Spectroscopy</b>  | 390 |
| 9.3.2          | NOEs in a Multi-Spin System  | 324 | 10.3.1    | The Problem of Convection                                   | 390 |
| 9.3.3          | Summary  | 329 | 10.3.2    | Calibrating Gradient Amplitudes                             | 397 |
| 9.3.4          | Applications   | 330 | 10.3.3    | Optimising Diffusion Parameters                             | 397 |
| 9.4            | <b>Transient NOEs</b>  | 335 | 10.3.4    | Hydrodynamic Radii and Molecular<br>Weights                 | 401 |
| 9.4.1          | Nuclear Overhauser Effect<br>Kinetics  | 335 | 10.4      | <b>Applications of Diffusion<br/>NMR Spectroscopy</b>       | 403 |
| 9.4.2          | Measuring Internuclear<br>Separations  | 336 | 10.4.1    | Signal Suppression  | 403 |
| 9.5            | <b>Rotating Frame NOEs</b>   | 337 | 10.4.2    | Hydrogen Bonding  | 405 |
| <b>PART II</b> | <b>PRACTICAL ASPECTS</b>   | 339 | 10.4.3    | Host–Guest Complexes  | 405 |
| 9.6            | <b>Measuring Transient NOEs: NOESY</b>   | 339 | 10.4.4    | Ion Pairing   | 408 |
| 9.6.1          | The 2D NOESY Sequence  | 339 | 10.4.5    | Supramolecular Assemblies                                   | 409 |
| 9.6.2          | 1D NOESY Sequences   | 346 | 10.4.6    | Aggregation   | 411 |
| 9.6.3          | Applications   | 349 | 10.4.7    | Mixture Separation  | 412 |
|                |  |     | 10.4.8    | Macromolecular Characterisation                             | 413 |

|   |     |   |     |
|---|-----|---|-----|
| <b>10.5 Hybrid Diffusion Sequences</b>                              | 414 | <b>12.4 Selective Excitation and Soft Pulses</b>  | 468 |
| 10.5.1 Sensitivity-Enhanced<br>Heteronuclear Methods                | 414 | 12.4.1 Shaped Soft Pulses   | 469 |
| 10.5.2 Spectrum-Edited Methods                                      | 415 | 12.4.2 Excitation Sculpting   | 473 |
| 10.5.3 Diffusion-Encoded Two-Dimensional<br>Methods (or 3D DOSY)    | 415 | 12.4.3 Chemical Shift Selective Filters   | 475 |
| References  | 418 | 12.4.4 DANTE Sequences  | 477 |
|   |     | 12.4.5 Practical Considerations   | 478 |
| <b>11 Protein–Ligand Screening by NMR</b>                           |     | <b>12.5 Solvent Suppression</b>   | 480 |
| <b>11.1 Introduction</b>  | 421 | 12.5.1 Presaturation  | 480 |
| <b>11.2 Protein–Ligand Binding Equilibria</b>                       | 422 | 12.5.2 Zero Excitation  | 482 |
| <b>11.3 Resonance Lineshapes and Relaxation<br/>    Editing</b>     | 424 | 12.5.3 Pulsed Field Gradients   | 483 |
| 11.3.1 $^1\text{H}$ Relaxation-Edited NMR                           | 426 | <b>12.6 Suppression of Zero-Quantum<br/>    Coherences</b>  | 486 |
| 11.3.2 $^{19}\text{F}$ NMR  | 428 | 12.6.1 The Variable-Delay Z-Filter  | 486 |
| 11.3.3 Paramagnetic Relaxation<br>Enhancement                       | 429 | 12.6.2 Zero-Quantum Dephasing   | 487 |
| <b>11.4 Saturation Transfer Difference</b>                          | 430 | <b>12.7 Heterogeneous Samples and Magic<br/>    Angle Spinning</b>  | 489 |
| 11.4.1 The STD Sequence<br>and Practicalities                       | 432 | <b>12.8 Hyperpolarisation</b>   | 491 |
| 11.4.2 Epitope Mapping by STD<br>and DIRECTION                      | 436 | 12.8.1 <i>Para</i> -Hydrogen–Induced<br>Polarisation  | 491 |
| 11.4.3 $K_D$ Measurement by STD                                     | 437 | 12.8.2 Dynamic Nuclear Polarisation   | 493 |
| <b>11.5 Water-LOGSY</b>   | 438 | References  | 496 |
| 11.5.1 The Water-LOGSY Sequence                                     | 440 | <b>13 Structure Elucidation and Spectrum<br/>    Assignment</b>   |     |
| 11.5.2 Water-LOGSY Practicalities                                   | 441 | <b>13.1 <math>^1\text{H}</math> NMR</b>   | 500 |
| <b>11.6 Exchange-Transferred Nuclear<br/>    Overhauser Effects</b> | 441 | <b>13.2 <math>^1\text{H}</math>–<math>^{13}\text{C}</math> Edited HSQC</b>                                | 501 |
| <b>11.7 Competition Ligand Screening</b>                            | 443 | <b>13.3 <math>^1\text{H}</math>–<math>^1\text{H}</math> COSY and Variants</b>                             | 503 |
| 11.7.1 Competitive Displacement                                     | 444 | 13.3.1 Double-Quantum<br>Filtered COSY  | 505 |
| 11.7.2 Reporter Ligand Screening                                    | 445 | <b>13.4 <math>^1\text{H}</math>–<math>^1\text{H}</math> TOCSY and Variants</b>                            | 506 |
| 11.7.3 $^{19}\text{F}$ FAXS   | 447 | 13.4.1 HSQC-TOCSY   | 508 |
| <b>11.8 Protein Observe Methods</b>                                 | 448 | <b>13.5 <math>^{13}\text{C}</math> NMR</b>  | 508 |
| 11.8.1 $^1\text{H}$ – $^{15}\text{N}$ Mapping                       | 448 | <b>13.6 HMBC and Variants</b>   | 510 |
| 11.8.2 $^1\text{H}$ – $^{13}\text{C}$ Mapping                       | 452 | 13.6.1 $^1\text{H}$ – $^{13}\text{C}$ HMBC  | 510 |
| 11.8.3 $^{19}\text{F}$ Mapping                                      | 452 | 13.6.2 $^{31}\text{P}$ and $^1\text{H}$ – $^{31}\text{P}$ HMBC  | 512 |
| References  | 454 | 13.6.3 $^1\text{H}$ – $^{13}\text{C}$ HMBC Again  | 513 |
|   |     | 13.6.4 $^{19}\text{F}$ and $^{19}\text{F}$ – $^{13}\text{C}$ HMBC   | 515 |
| <b>12 Experimental Methods</b>                                      |     | <b>13.7 Nuclear Overhauser Effects</b>  | 517 |
| <b>12.1 Composite Pulses</b>  | 457 | 13.7.1 2D NOESY   | 517 |
| 12.1.1 A Myriad of Pulses   | 459 | 13.7.2 1D NOESY   | 521 |
| 12.1.2 Inversion Versus Refocusing                                  | 460 | 13.7.3 1D $^{19}\text{F}$ HOESY   | 522 |
| <b>12.2 Adiabatic and Broadband Pulses</b>                          | 461 | <b>13.8 Rationalization of <math>^1\text{H}</math>–<math>^1\text{H}</math> Coupling<br/>    Constants</b> | 523 |
| 12.2.1 Common Adiabatic Pulses                                      | 462 | <b>13.9 Summary</b>   | 525 |
| 12.2.2 Broadband Inversion Pulses: BIPs                             | 464 |   |     |
| <b>12.3 Broadband Decoupling and Spin Locking</b>                   | 465 | Appendix  | 527 |
| 12.3.1 Broadband Adiabatic Decoupling                               | 467 | Subject Index   | 531 |
| 12.3.2 Spin Locking   | 468 |   |     |